SLEEP ELECTROPHYSIOLOGICAL CHANGES IN CHILDREN AND ADOLESCENTS IN RELATION TO CEREBRAL OXYGENATION AND AFTER INTENSIVE WORKING MEMORY TRAINING

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Content

1. Summary

Sleep is a behavioral state that disengages an individual from the environment (Carskadon & Dement, 2011). Between vigilance states, that is, between wake, sleep and distinct sleep stages, the level of neuronal activity changes, which can be observed on the scalp by electroencephalography (EEG). Since neuronal activity is coupled to oxygen demand, these changes are also reflected in altered cerebral blood perfusion. The need for sleep is homeostatically regulated: it increases during periods of wakefulness and dissipates during sleep. This regulatory process is reflected by slow wave activity (SWA, EEG power between 0.5 to 4.5 Hz). Similar to sleep need, and as a function of the duration of the preceding wakefulness, SWA is highest at sleep onset and decreases during sleep (Achermann & Borbély, 2011).

One goal of this project was to study vigilance state transitions in relation to cerebral blood perfusion. Next, the aim was to examine short- and long-term effects of intensive working memory on cognitive performance. Thirdly, the question was pursued if the intensive working memory training would lead to changes in SWA. Finally, cerebral perfusion was assessed in deep sleep. The studies were performed in a group of young subjects aged between 10 and 16 years: in this age range, SWA is maximal (Feinberg, Higgins, Khaw, & Campbell, 2006).

As the first hypothesis, this work proposes that energy demand at vigilance state transitions may be measured by applying near-infrared spectroscopy (NIRS). By recording hemodynamic changes with NIRS, the correlation between oxygen consumption changes and metabolic turnover at transitions from wake to sleep and during sleep were studied. Among other measures, tissue oxygen saturation (StO_2) was recorded. As presented in section 4.1, StO_2 changes were found to correspond to changes in vigilance states as determined by EEG. For instance, from wakefulness to deep sleep, when neuronal activity decreases, also StO_2 decreased, presumably reflecting a reduction in brain oxygen consumption. The results show that StO_2 changes measured by NIRS coupled to the EEG findings may reflect metabolic changes at vigilance transitions.

Secondly, this study aimed to manipulate neuronal activity by intensive learning. It was suggested that working memory training may be suitable for this, since it has been shown that working memory training serves as a tool to improve cognitive skills (Klingberg, 2010). According to the study hypothesis, the applied working memory training (visuospatial n-back) would be considered effective, if the expected cognitive performance improvement persisted over several months after the training period (see section 4.2). Furthermore, taking into account reports of working memory training effects on untrained tasks (Jaeggi, Buschkuehl, Jonides, & Perrig, 2008), performance in multiple cognitive tasks was assessed. In the study presented here, it was shown that the training group improved their performance in an un-

trained working memory task (auditory n-back) immediately and also several months after the training period. Regarding these short-term and long-term effects, it may be concluded that the working memory training seemed to be beneficial for the young study population.

As a third hypothesis, it was proposed that the working memory training benefits are reflected in changes in sleep SWA. There is accumulating evidence that sleep and especially slow waves are associated with learning processes and synaptic plasticity. SWA has been shown to reflect changes in synaptic strength (e.g., Riedner et al., 2007). Consequently, upon manipulation of synaptic strength during wakefulness, e.g., by learning, synaptic plasticity-induced changes in SWA were observed in subsequent sleep (for review, see Lustenberger & Huber, 2012). For instance, it was shown in adult subjects that after intense learning in the evening, slow waves were locally altered in the subsequent night (e.g., Huber, Ghilardi, Massimini, & Tononi, 2004). So far, studies have focused on investigating the immediate effects of learning on SWA. In this work (see section 4.3), the aim was to investigate the association between sleep slow waves and learning over several weeks. To this end, sleep high-density electroencephalography (HD EEG) was recorded before and after working memory training. This revealed an increase in SWA after three weeks of training in three left frontoparietal derivations. The increase correlated positively with the immediate and long-term performance in the auditory n-back task. Thus, the data indicate that local changes in sleep SWA may reflect learning-induced synaptic plasticity after several weeks, in a region known to be involved in working memory processes.

However, it is hypothesized that SWA not only reflects, but also functionally contributes to synaptic plasticity. It was shown that during wake, synaptic potentiation processes dominate (Vyazovskiy, Cirelli, Pfister-Genskow, Faraguna, & Tononi, 2008), which is an expensive process for the brain, e.g., in terms of consumed energy resources. It was suggested that sleep and especially slow waves in deep sleep are necessary for counterbalancing these limitations by a proportional reduction in synaptic strength (downscaling; Tononi & Cirelli, 2006). Thus, in a last study (see section 4.4), StO_2, as a correlate for energy consumption of the brain, was measured during sleep. As a result, StO_2 increased during sleep, which may indicate that the availability of oxygenated blood increases while SWA decreases. In line with the synaptic homeostasis hypothesis, the increase in StO_2 suggests a relationship with an increased supply of energy after sleep.

In summary, this work reveals that changes in neuronal activity at vigilance state transitions and during sleep may be investigated by measuring hemodynamic changes over night, using NIRS alongside to HD EEG in a young population. The data indicate that NIRS may serve as a tool to further explore the role of slow waves in energy restoration. Furthermore, it was found that intensive working memory training increased cognitive performance short- and long-term in children and adolescents. For the first time, an increase in local SWA in relation to training-induced cognitive

performance changes is shown, presumably reflecting synaptic plastic changes. Overall, these findings contribute to the evidence that SWA may be used as a tool to map learning-induced plasticity.

2. Zusammenfassung

Schlaf ist ein Zustand in dem ein Individuum von Umwelteinflüssen losgelöst wird (Carskadon & Dement, 2011). Die neuronale Aktivität unterscheidet sich zwischen Wachsamkeitszuständen (engl.: *vigilance states*), d.h. zwischen Wach und Schlaf und zwischen den Schlafstadien, und kann mittels Elektroenzephalographie (EEG) beobachtet werden. Da neuronale Aktivität an den Bedarf von Sauerstoff gekoppelt ist, lassen sich diese Übergänge auch anhand einer veränderten zerebralen Durchblutung erkennen. Das Bedürfnis zu schlafen ist homöostatisch reguliert: während der Wachperiode steigt es an, und im Schlaf wird es abgebaut. Dieser geregelte Prozess wird durch das Auftreten von langsamen Wellen (*slow wave activity*, SWA; EEG Signalleistung zwischen 0.5 und 4.5 Hz) widerspiegelt: In Abhängigkeit des Schlafbedürfnisses, und somit als Funktion der vorangehenden Wachdauer, ist die SWA bei Schlafbeginn am höchsten, und nimmt während des Schlafs wieder ab (Achermann & Borbély, 2011).

Ein Ziel dieses Projekts war es, die neuronale Aktivität bei Übergängen zwischen den Wachsamkeitszuständen im Zusammenhang mit zerebraler Durchblutung zu beobachten. In einer weiteren Studie war es das Ziel, kurz- und langfristige Effekte eines intensiven Arbeitsgedächtnistrainings auf die kognitive Leistung zu untersuchen. Drittens wurde der Frage nachgegangen, ob als Folge des Arbeitsgedächtnistrainings Veränderungen der SWA zu beobachten sind. Schliesslich wurde auch die zerebrale Durchblutung im Tiefschlaf gemessen. Alle Studien wurden in einer Gruppe junger Probanden im Alter zwischen 10 und 16 Jahren durchgeführt; in diesem Alter ist die SWA maximal (Feinberg et al., 2006).

Die erste Hypothese dieser Arbeit besagt, dass Veränderungen des Energiebedarfs beim Übergang zwischen Wachsamkeitszuständen durch den Einsatz von Nah-Infrarot Spektroskopie (NIRS) beobachtet werden können. Mittels NIRS wurden solche hämodynamische Veränderungen gemessen. Diese Änderungen widerspiegeln Unterschiede im Sauerstoffbedarf, einem Korrelat für metabolischen Umsatz. Nebst anderen Grössen wurde die Gewebesauerstoffsättigung (StO_2) erfasst. Wie in Abschnitt 4.1 vorgestellt, wurden StO_2 Veränderungen in Übereinstimmung mit den mittels EEG bestimmten Veränderungen des Wachsamkeitszustands gefunden. Beispielsweise nahm beim Übergang vom Wachzustand zum Tiefschlaf, bei welchem die neuronale Aktivität abnimmt, auch der StO_2 Wert ab, was auf eine Abnahme des Sauerstoffkonsums im Gehirn hinweist. Demnach zeigen die Resultate, dass der mittels NIRS gemessene Wert für StO_2 potentiell Aussagen über metabolische Veränderungen beim Übergang von Wachsamkeitszuständen ermöglicht.

Das zweite Ziel dieser Studie war es, die neuronale Aktivität durch intensives Lernen zu manipulieren. Es wurde angenommen, dass ein Arbeitsgedächtnistraining dafür geeignet ist, da gezeigt worden war, dass durch Arbeitsgedächtnistraining kognitive

Fähigkeiten verbessert werden können (Klingberg, 2010). In dieser hier präsentierten Studie wurde das Arbeitsgedächtnistraining (visuell-räumliches n-back) als effektiv betrachtet, wenn der (erwartete) Anstieg in der kognitiven Leistung auch über mehrere Monate nach der Trainingsperiode anhielt (siehe Abschnitt 4.2). Des Weiteren, unter Berücksichtigung von Berichten über die Effekte eines Arbeitsgedächtnistrainings auf nicht trainierte Aufgaben (z. B. Jaeggi et al., 2008), wurde die Leistung in mehreren kognitiven Tests gemessen. Damit konnte gezeigt werden, dass die Trainingsgruppe ihre Leistung in einer untrainierten Arbeitsgedächtnisaufgabe (auditiver n-back) sowohl unmittelbar wie auch mehrere Monate nach der Trainingsperiode verbesserte. In Anbetracht dieser kurz- und langfristigen Effekte wurde der Schluss gezogen, dass das praktizierte Arbeitsgedächtnistraining für eine junge Population förderlich ist.

In einer dritten Hypothese wurde vorgeschlagen, dass die Effekte des Arbeitsgedächtnistrainings zu Veränderungen in der SWA führen, da es zunehmend Hinweise darauf gibt, dass Schlaf, und im Speziellen die langsamen Wellen, mit Lernprozessen und synaptischer Plastizität assoziiert sind. SWA reflektiert Veränderungen der synaptischen Stärke (z. B. Riedner et al., 2007). Folglich können durch Manipulation der synaptischen Stärke im Wachzustand, zum Beispiel durch Lernen, synaptische plastische Veränderungen mittels Veränderungen der SWA in der darauffolgenden Schlafperiode beobachtet werden (siehe Review von Lustenberger & Huber, 2012). Es konnte zum Beispiel gezeigt werden, dass bei Erwachsenen intensives Lernen am Abend die SWA in der darauffolgenden Nacht lokal verändert (z. B. Huber et al., 2004). Bis jetzt konzentrierte man sich bei solchen Studien auf die unmittelbaren Einflüsse des Lernens auf die SWA. In dieser Arbeit (siehe Abschnitt 4.3) war es das Ziel, die langsamen Wellen im Tiefschlaf in Zusammenhang mit Lernen über mehrere Wochen zu untersuchen. Um dies zu erreichen wurde während des Schlafs hochauflösendes (*high-density*, HD) EEG vor und nach dem Arbeitsgedächtnistraining aufgezeichnet. Nach drei Wochen Training wurde eine Zunahme der SWA in drei linken frontoparietalen Elektroden gefunden. Diese lokale Zunahme korrelierte positiv mit der unmittelbaren und langfristigen Leistung in der auditiven n-back Aufgabe. Demzufolge scheinen die Resultate darauf hinzuweisen, dass die lokale Veränderung der SWA durch Lernen induzierte, synaptische Plastizität reflektiert; dies in einer Region, die bekanntermassen in Arbeitsgedächtnisprozesse involviert ist.

Es wird jedoch angenommen, dass die SWA nicht nur synaptische Plastizität reflektiert, sondern auch einen funktionellen Beitrag dazu leistet. Es konnte gezeigt werden, dass im Wachzustand synaptische Potenzierung dominiert (Vyazovskiy et al., 2008), was einen energieintensiven Prozess für das Hirn darstellt. Es wird angenommen, dass Schlaf, und im Speziellen die langsamen Wellen des Tiefschlafs, wichtig sind, um diesem Prozess entgegen zu wirken indem die synaptische Stärke wieder reduziert wird (*downscaling*; Tononi & Cirelli, 2006). Deshalb wurde in der letzten Studie (siehe Abschnitt 4.4) StO_2 als ein Korrelat für den Energieverbrauch des Ge-

hirns über Nacht gemessen. Es wurde eine Zunahme der StO_2 im Schlaf beobachtet, was darauf hinweist, dass die Verfügbarkeit von mit Sauerstoff angereichertem Blut zunimmt während die SWA abnimmt. Die Zunahme an StO_2 deutet darauf hin, dass nach dem Schlaf die Verfügbarkeit an Energie höher ist, wie es von der Synaptischen Homöostase Hypothese vorausgesagt wird (Tononi & Cirelli, 2006).

Zusammenfassend zeigt diese Arbeit, dass Veränderungen der neuronalen Aktivität beim Übergang von Wachsamkeitszuständen sowie während des Schlafes und die damit verbundenen hämodynamischen Prozesse mittels NIRS und gleichzeitiger EEG in einer jungen Population gemessen werden können. Zudem weisen die Daten darauf hin, dass NIRS eine geeignete Methode zu sein scheint, um die Rolle der langsamen Wellen im Zusammenhang mit dem Energieverbrauch des Gehirns weiter zu untersuchen. Des Weiteren wurde durch intensives Arbeitsgedächtnistraining die kognitive Leistung bei Kindern und Jugendlichen insbesondere langfristig verbessert. Ausserdem wurde zum ersten Mal eine lokale Zunahme der SWA in Zusammenhang mit einer durch Training induzierten Verbesserung der kognitiven Leistung gezeigt, was möglicherweise synaptische Plastizität widerspiegelt. Insgesamt liefert diese Studie einen weiteren Hinweis darauf, dass SWA geeignet ist, um durch Lernen induzierte Plastizität abzubilden.

3. Introduction

Sleep is a behavioral phenomenon that has been described in many species (Tobler, 2000). The behavioral characteristics that constitute sleep are driven by the brain. From wakefulness to sleep, neuronal activity changes drastically (Steriade, 2006). These changes have been studied in animal models as well as in humans. Despite extensive investigation, the biological function of sleep remains unclear. One suggestion is that sleep serves to preserve energy. Another aspect of sleep is its role in learning. In this work, two approaches are presented to investigate these aspects.

Using electroencephalography (EEG) measured on the human scalp, the different vigilance states, i.e., wakefulness and sleep and sleep stages, are separated by distinct characteristics. These are described as in the very first section of this work. The vigilance states emerge due to changes in neuronal activity, which was mainly explored in animals. Electrophysiological changes in neuronal activity are also affecting brain metabolism and energy consumption. Here, near-infrared spectroscopy (NIRS) was used as a tool to measure non-invasively changes in blood oxygen concentrations that are related to metabolism.

Another aspect of sleep is its role in learning. There is increasing evidence that sleep reflects learning-induced synaptic plasticity. The mechanisms of synaptic plasticity are the basis for learning processes. In humans, inducing learning-related synaptic plasticity may be assessed by using an intensive cognitive training, such as working memory training. Changing neuronal activity during wake by applying a learning paradigm was shown to alter synaptic connections. The modification of these connections in turn results in changes in slow waves during deep sleep.

3.1. Sleep characteristics

In this first chapter, basic definitions to distinguish sleep from wake as well as mechanisms of sleep-wake regulation are presented.

3.1.1. Vigilance states

On behavioral level, normal human sleep is hardly dissociable from resting wakefulness because both are characterized by closed eyes and calm breathing. However, sleep is a brain phenomenon driving the body to rest (Hirshkowitz, 2004). It is defined as a reversible state of “perceptual disengagement from the environment” and “reduced responsiveness to external stimuli from the environment” (Carskadon & Dement, 2011). With EEG measurements on the scalp, brain activity characteristics (e.g., amplitude and frequency) during sleep and its various states are dissociable from those observed during wake. The rules for scoring the different vigilance states were first established by Rechtschaffen and Kales (Rechtschaffen & Kales, 1968). In

this work, visual scoring of different sleep states within 20-second-epoch windows is based on the novel rules described in the manual of the American Academy of Sleep Medicine (AASM; Iber, Sonia Ancoli-Israel, Chesson, Quan, & eds, 2007).

With these rules, vigilance states are determined based on shape and amplitude of the EEG signal, enabling the discrimination of wakefulness and different sleep stages. Wake is dominated by fast rhythms in beta (15 to 30 Hz) and gamma (30 to 60 Hz) bands. When having the eyes closed, alpha rhythm (8 to 13 Hz) may be observed. In the transition from wake to sleep (non-rapid eye movement (NREM) sleep stage 1), eye movements slow down and the alpha rhythm vanishes. After sleep stage 1, sleep deepens from rather light sleep (NREM sleep stage 2), characterized by sleep spindles and K-complexes, to deep sleep, which is dominated by slow waves (amplitude higher than 75 µV, frequency 0.5 to 2 Hz, NREM sleep stage 3). After a period of NREM sleep, rapid eye movement (REM) sleep is observed, with comparable brain activity as during wake but accompanied by muscle atonia and rapid eye movements. Each sleep cycle including NREM and REM sleep lasts approximately 80 to 100 minutes and repeats several times within a sleep period (Carskadon & Dement, 2011). The distribution of sleep stages within a sleep period alters with age, showing the most striking changes in sleep depth: with increasing age, percentage of slow wave sleep decreases and wake arousals as well as percentage of NREM sleep stage 1 and 2 increases (Ohayon, Carskadon, Guilleminault, & Vitiello, 2004). The next section discusses the neuronal activity underlying these vigilance states.

3.1.2. Molecular basis for vigilance states and the generation of sleep oscillations

The transitions between vigilance states depend on the neuromodulatory system originating in the brain stem, which includes two ascending systems that innervate the thalamus and the hypothalamus (Saper, Chou, & Scammell, 2001; Saper, Scammell, & Lu, 2005). The first ascending projection to the thalamus derives from caudal midbrain and rostral pons, the cholinergic pedunculopontine and laterodorsal tegmental nuclei (PPT-LDT). During wake and REM sleep, the PPT-LDT neurons fire rapidly whereas at NREM sleep, the firing rate reduces. Second, monoaminergic cell groups within the PPT-LDT, such as noradrenergic locus coeruleus and serotonergic raphe nuclei, send their axons via the histaminergic tuberomammillary nucleus (TMN) to the lateral hypothalamus. These cell groups fire at high rate during wake, at lower rate during NREM sleep and cease firing at REM sleep. Thus, during REM sleep, monoaminergic input is reduced (Saper et al., 2005). Overall, external sensory inputs from the brainstem over thalamus to the neocortex are markedly reduced during sleep, leading to the disconnection from the environment and enabling the generation of sleep oscillations.

In animals, origin and generation of sleep oscillations, which are specific for different vigilance states, were investigated on cellular and molecular level. The waking

brain activity is characterized by tonic depolarization of cortical cells and irregular firing (Vyazovskiy et al., 2011). From wake to NREM sleep, mean firing rate decreases about 35 % and increases again from NREM to REM sleep (Vyazovskiy et al., 2009). One defining feature of oscillations occurring during NREM sleep is the prolonged hyperpolarization of thalamic and cortical neurons which prevents the transmission of afferent, wake-inducing signals from the brain stem to the cortex (Steriade & Timofeev, 2003). From sleep stage 1 over stage 2 to stage 3, sleep deepens progressively which is associated with increased hyperpolarization of thalamocortical cells.

Intracellular measurements in animals revealed that slow waves measured by scalp EEG reflect slow oscillations (frequency lower than 1 Hz) generated in neocortical neurons (Steriade, Nunez, & Amzica, 1993b). The slow oscillation reflects the alteration between up and down states (see Fig. 3.1, top). The up state represents prolonged neuronal depolarization of the membrane potential. This is mediated e.g., by non-*N*-methyl-D-aspartate (non-NMDA) elicited excitatory postsynaptic potentials (EPSP), voltage-dependent persistent Na^+ current, in local circuit cells within the cortex (Steriade, Nunez et al., 1993b). The depolarizing phase is followed by a long phase (lasting up to 1 s) of silence, the hyperpolarized down state (Steriade, Nunez et al., 1993b). It relies on disfacilitation (i.e. the removal of synaptic input), involving activation of K^+ channels (Contreras & Steriade, 1995; Contreras, Timofeev, & Steriade, 1996). From up to down state, extracellular Ca^{2+} concentration decreases, which modulates synaptic transmission (Massimini & Amzica, 2001).

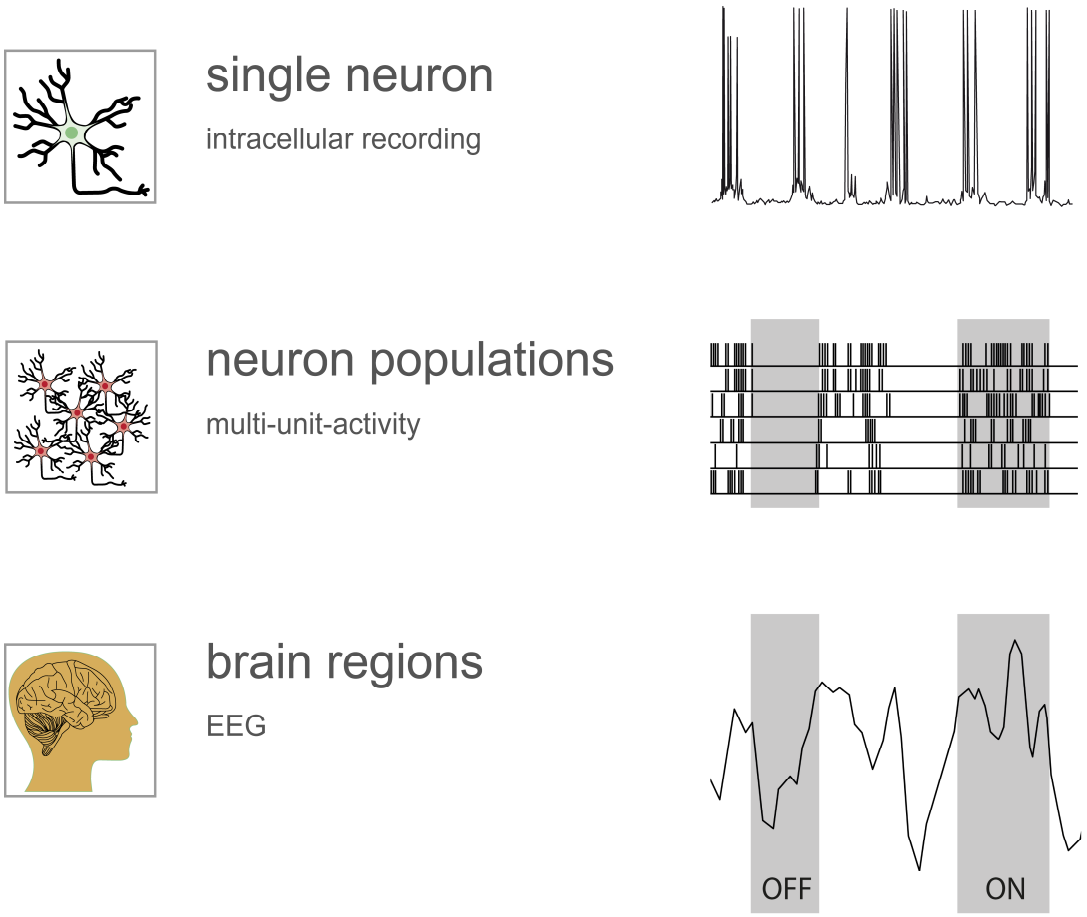

Figure 3.1. Top: Measured in single cortical neurons, slow oscillations (< 1 Hz; here: intracellular recordings in a cat; Steriade, Nunez et al., 1993b) consist of depolarizing up state and a subsequent phase of silence (hyperpolarizing down phase, membrane polarization -68 mV). Middle: The firing within population of neurons (displayed here in six neurons) is highly synchronous during deep sleep. Bottom: This synchronous pattern of ON and OFF states (multi-unit recording in neuronal populations, Vyazovskiy et al., 2009), is detected as slow waves, measured by scalp EEG (Achermann & Borbély, 2011).

It was suggested that the neocortex is sufficient to generate slow oscillations, because they are observed in thalamectomized animals (Steriade, 2006; Steriade, Nunez, & Amzica, 1993a) and cortical slices (Sanchez-Vives & McCormick, 2000) and are absent in decorticated animals (Timofeev & Steriade, 1996). Extracellular multi-unit activity measurements show that the up state (ON phase) and down state (OFF) are highly synchronous in large population of neurons (Fig. 3.1, middle; Steriade, McCormick, & Sejnowski, 1993; Steriade, Timofeev, & Grenier, 2001; Vyazovskiy et al., 2009), leading to slow waves which can be measured on the scalp using surface EEG (Contreras & Steriade, 1995). The near-synchronicity of transition from spiking (ON phase) of neuronal population to subsequent silence (OFF phase) leads to increased amplitude of slow waves (Fig. 3.1, bottom), depending on the strength of synaptic connections between neurons (e.g., Esser, Hill, & Tononi, 2007; Riedner et al., 2007; Vyazovskiy, Riedner, Cirelli, & Tononi, 2007). It is therefore assumed

that changes in synaptic strength are reflected in amplitude and slope changes of slow waves (for more details see section 3.2.1).

Notably, different oscillations occur simultaneously, whereby slow oscillations play a major role in grouping those rhythms (Steriade, 2006). For example sleep spindles (of a frequency between 10 and 15 Hz) are triggered by the synchronous firing of cortical slow waves during their depolarizing phase (e.g., Amzica & Steriade, 1997). Sleep spindles appear as an oscillation of waxing and waning amplitude lasting 1 to 3 s (for review, see Luthi, 2013). They are generated and synchronized within the thalaomocortical network (Luthi, 2013; Steriade, McCormick et al., 1993). The pacemaker of spindles is the thalamic reticular nucleus (RE). Gamma-aminobutyric acid (GABA)-ergic afferents from the RE hyperpolarize the thalamocortical (TC) cells (Fuentealba & Steriade, 2005), leading to inhibitory postsynaptic potentials (IPSP; Steriade, McCormick et al., 1993). Due to ionic currents that are de-inactivated by the IPSP, the TC cells exhibit a rebound spike, resulting in a burst of action potentials (Cueni et al., 2008). This burst excites cortical neurons, which in turn feedback to the RE neurons via excitatory afferents (Steriade, McCormick et al., 1993). Cortical neurons initiate and synchronize spindles via excitatory afferents to the RE and TC neurons (Sanchez-Vives & McCormick, 2000). During the waxing phase, the synchronous burst of TC cells is further amplified in cortical layers, leading to the sleep spindles detectable by scalp EEG (Dijk, Hayes, & Czeisler, 1993; Luthi, 2013). The waning phase of spindles is mediated by intrinsic properties of RE and TC cells, that finally exert network desynchronization and thereby spindle termination (Luthi, 2013; Timofeev & Chauvette, 2011).

In order to quantitatively analyze the oscillations during sleep (e.g., sleep slow waves and spindles), fast fourier transformation (FFT) is applied to transform the time signal into the frequency domain (Achermann, 2009). The FFT estimates the oscillations within a finite time window according to frequency. The duration ([ms], within the signal window) and amplitude [µV] of the oscillation determines the power density [$\mu V^2\ Hz^{-1}$] for specific oscillation frequencies. This allows analyzing data based on specific frequency bands related to sleep. Specifically, the power of slow waves (in the frequency band between 0.5 and 4.5 Hz), called slow wave activity (SWA) and in spindles within frequency bands between 12 and 15 Hz (sigma range) are of interest in sleep research.

As introduced above, neuronal activity changes from wakefulness to sleep. These changes are associated to variations in metabolic turnover. This is discussed in the following section.

3.1.3. Metabolic correlates for neuronal activity at different vigilance states

In humans, EEG is an established method to measure postsynaptic potentials from pyramidal neurons in the cortex. The summed electrical potentials of neuronal popu-

lations are recorded with high temporal resolution by electrodes on the scalp (unit: [V]; Achermann, 2009). The differences in neuronal activity between wake and sleep affect energy consumption of the brain (Maquet, 1995). Specifically, wakefulness which is dominated by a high firing rate of neurons is linked to higher energy demands than slow wave sleep with its characteristic phases of silence (Maquet, 1995; Nofzinger et al., 2002; Vyazovskiy & Harris, 2013). It has been shown early that energy demand is linked to tissue oxygen consumption. Specifically, changes in neuronal activity are associated with (localized) changes in cerebral perfusion (Roy & Sherrington, 1890). For activated brain areas, oxygen and glucose consumption increases (Blomqvist et al., 1994; Seitz & Roland, 1992). Neuronal activity is correlated to an increased inflow of oxygenated blood into activated brain areas, which is achieved by an active control of the flow resistance of arterioles and capillaries (Iadecola, Yang, Ebner, & Chen, 1997): this process is termed neurovascular coupling (for review, see Iadecola, 2004; Raichle & Mintun, 2006). Thus, localized changes in blood perfusion may serve as a measure for changes in tissue metabolism.

During sleep, several methods are employed to assess blood perfusion (changes) with spatial resolution including functional magnetic resonance imaging (fMRI) and positron emission tomography (PET; Dang-Vu, 2012; Dang-Vu et al., 2010). fMRI acquires the blood oxygen level dependent (BOLD) signal. The BOLD signal is generated by changes in the concentration of deoxygenated hemoglobin, which is, unlike oxygenated hemoglobin, paramagnetic (Logothetis & Wandell, 2004). By means of PET, one can measure concentration changes of radioactive tracers in the blood. This enables the measurement of localized blood perfusion changes. Kaufmann et al. (Kaufmann et al., 2006) reported localized decreases of the BOLD signal during NREM sleep compared to wake. Similar results were found with PET (Braun et al., 1997). Braun et al. (Braun et al., 1997) reported a deactivation in brain stem during sleep, which is believed to represent sleep related reduced firing in this structure. These neuroimaging methods complement the EEG well because they provide a correlate measure for metabolic activity with high spatial resolution. However, fMRI and PET have some disadvantages, particularly in sleep research: continuous overnight measurement in the scanner is demanding and may result in impaired sleep quality (Kaufmann et al., 2006). Both methods are expensive, and in addition, MRI is noisy and PET is an invasive procedure since it requires the injection of radioactive tracer substances (Jedidi et al., 2012).

One possible approach to monitor metabolic activity, yet avoiding above mentioned short-comings, is the application of near-infrared spectroscopy (NIRS). NIRS non-invasively measures perfusion-dependent light attenuation of the tissue. Basically, NIRS sensors send light through tissue by a light source (emitter) which is scattered back to a detector (Wolf et al., 2008). Usually, the concentrations of oxyhemoglobin ([O_2Hb]), deoxyhemoglobin ([HHb]) and total hemoglobin ([tHb]), which is the sum of the former two variables, are measured in tissue (see Fig. 3.2). For brain activity

measurements, cerebral tissue is targeted. However, it should be considered that besides brain tissue, also scalp, scull, meninges and cerebral spinal fluid contribute to overall light attenuation. Since NIRS does not provide anatomical information, the contribution of these attenuators remains unknown but usually it is assumed that it remains constant over time. Consequently, assumptions must be taken, e.g., about the mean optical path length (Duncan et al., 1996), to calculate relative changes of [O_2Hb], [HHb] and [tHb] over time by applying the modified Beer-Lambert law (Delpy, Cope, Van der Zee, Wray, & Wyatt, 1988). NIRS sensors monitor superficial cortical structures only locally and not as a whole, as opposed to PET and fMRI.

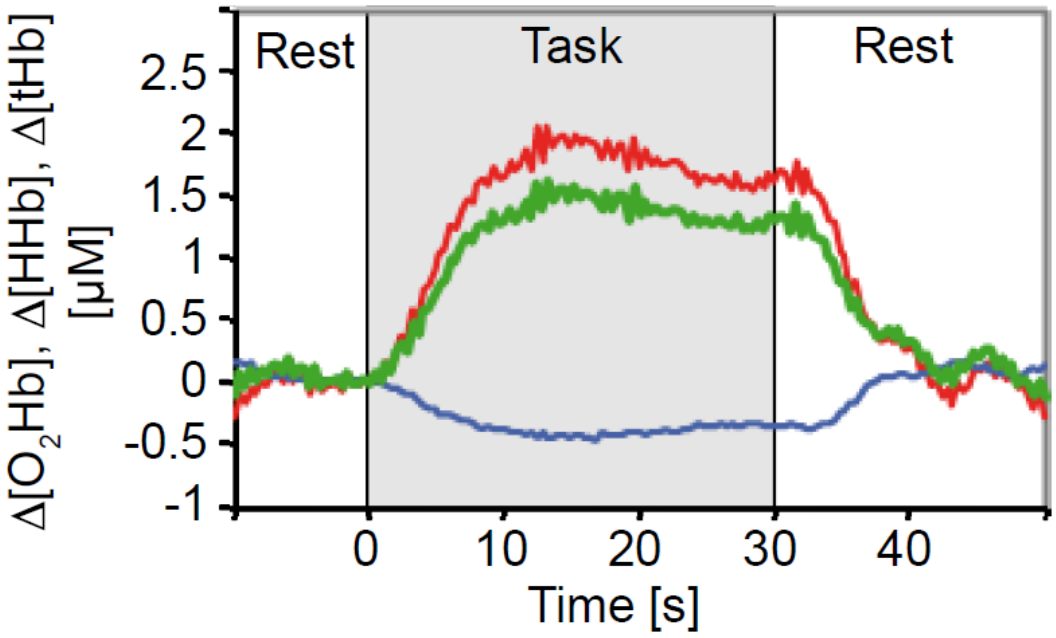

Figure 3.2: Typical hemodynamic response to a visual stimulus (gray square) measured over the occipital cortex. Increased oxygen demand results in increased inflow of oxygenated arterial blood, mediated by neurovascular coupling. Consequently, the concentration in O_2Hb (red trace) increases. Despite the increased oxygen consumption, HHb concentration (blue trace) decreases during activation, because of the dominant influence of increased perfusion with arterial (i.e. oxygenated) blood. Total hemoglobin ([O_2Hb] + [HHb]) is depicted in green (picture credit to Martin Wolf).

NIRS measures were shown to correlate with the BOLD signal (Strangman, Boas, & Sutton, 2002) and NIRS can be applied concurrently to sleep EEG (e.g., Uchida-Ota, Tanaka, Sato, & Maki, 2008). These first applications of NIRS during sleep revealed changes in oxygen consumption at distinct vigilance states. Compared to light sleep, REM sleep and wakefulness, hemodynamic processes were reduced during slow wave sleep (Näsi et al., 2011). Further, transitions between vigilance states could be observed (Näsi et al., 2011; Uchida-Ota et al., 2008). For example, [O_2Hb] increased from sleep stage 2 to 1 and decreased from sleep stage 1 to 2 (Uchida-Ota et al., 2008).

3.1.4. Sleep regulation

The alternating phases of sleep and wake are not random. Rather, the propensity to sleep is guided by two different processes: process S and C. Process S refers to the homeostatic regulation of sleep and wake, and thus, takes prior sleep-wake history into account (Achermann & Borbély, 2011). A marker for sleep homeostasis is sleep intensity, measured by slow wave activity (SWA) during NREM sleep. The time course of SWA, i.e. its build-up during wake and decline during sleep, reflects process S and parallels the changes in sleep need (Achermann & Borbély, 2011). Accordingly, a nap during the day reduces SWA at night (Werth, Dijk, Achermann, & Borbely, 1996) whereas prolonged wake (e.g., sleep deprivation or restriction) increases SWA in the following sleep episode (Achermann & Borbély, 2011; Borbély, Baumann, Brandeis, Strauch, & Lehmann, 1981; Dijk et al., 1993).

Sleep propensity was also shown to depend on time of day, that is the circadian rhythm, which is referred to as process C (Borbely, 1982). Circadian rhythms are driven by the circadian clock which is associated to an anatomical structure, the suprachiasmatic nucleus (SCN), located in the anterior hypothalamus (Klein, Moore, & Reppert, 1991; Moore & Eichler, 1972). The SCN generates and synchronizes circadian rhythms (Kramer et al., 2001; C. Liu, Weaver, Strogatz, & Reppert, 1997), and is crucial for the entrainment of these rhythms to external Zeitgebers such as light (Franken & Dijk, 2009; Gronfier, Wright, Kronauer, & Czeisler, 2007). The circadian process results in a sinusoidal alteration of behavioral and physiological markers with a period length of approximately 24 hours, influencing the rest-activity pattern (Czeisler & Gooley, 2007). Examples for such markers are core body temperature (Krauchi & Wirz-Justice, 1994) and melatonin concentration (Czeisler et al., 1999). The interaction between processes S and C was established with the two process model (Borbely, 1982): with increasing time awake, homeostatic sleep drive (or sleep propensity) accumulates (process S). Consequently, cognitive performance and alertness would decrease, which is countered by process C, thereby ensuring sustained performance level during time awake (Achermann & Borbély, 2011; Cajochen, Khalsa, Wyatt, Czeisler, & Dijk, 1999). Thus, also during prolonged wakefulness, cognitive performance and alertness do not steadily decrease but alternate in accordance with the circadian rhythm (Wyatt, Ritz-De Cecco, Czeisler, & Dijk, 1999). With forced desynchrony (FD) protocols or sleep deprivation, the two processes may be disentangled (Dijk & Czeisler, 1994, 1995; Lavie, 2001). This revealed that SWA does not seem to be modulated by process C (Dijk & Czeisler, 1995).

3.2. Sleep and plasticity

In the sections above, neuronal activity from wake to sleep was described. One main feature of wake is its association to learning processes, which induces plasticity and alters neuronal activity. Plasticity refers to a process of behavioral modification, e.g., learning and acquiring new skills, and is manifested by the intrinsic property of the brain to undergo plastic changes due to external and internal stimuli (Pascual-Leone, Amedi, Fregni, & Merabet, 2005). In the following section, basic mechanisms of synaptic plasticity as well as working memory training as a potential tool to assess learning and induce plasticity are presented. In the last section, a potential function of sleep related to learning is introduced. Finally, a link between learning-induced synaptic plasticity and sleep is presented.

3.2.1. Molecular mechanisms of synaptic plasticity

The cellular and molecular mechanisms of neuronal plasticity are based on functional (within several minutes to hours) and structural modifications (observable after days and weeks) of synapses, the connective structure between neurons (Buonomano & Merzenich, 1998; Soderling & Derkach, 2000).

Functionally, synaptic transmission changes due to learning. Based on the Hebbian learning rule (Hebb, 1949), synapses are strengthened short-term (i.e. within some minutes) or over several days and weeks by almost coincident activation of the pre- and postsynapses (long-term potentiation, LTP). Accordingly, synapses are weakened if releasing presynaptic transmitters does not elicit a postsynaptic action potential but rather, if postsynaptic firing occurs before presynaptic firing (long-term depression, LTD; Benington & Frank, 2003). Thus, the relative timing of pre- and postsynaptic firing is important for plasticity (spike timing dependent plasticity, STDP; Roberts & Bell, 2002) and the temporal correlation of the firing activity determines synaptic transmission efficacy (Turrigiano, 1999; Turrigiano, Leslie, Desai, Rutherford, & Nelson, 1998).

LTP is induced by glutamate which is released from the presynapse into the synaptic cleft and activates postsynaptic *N*-methyl-D-aspartate (NMDA) glutamate receptor (Elgersma & Silva, 1999). This process increases intracellular [Ca^{2+}] in the dendritic spine which elicits postsynaptic response and finally leads to postsynaptic potentiation (Collingridge, Isaac, & Wang, 2004). Also other activity-dependent synaptic transmissions result in Ca^{2+} influx into the dendritic spines (Hering & Sheng, 2001), e.g., by voltage-gated Ca^{2+} channels (Benington & Frank, 2003; Chetkovich, Gray, Johnston, & Sweatt, 1991). During the early phase of LTP (E-LTP), the postsynaptic potentiation (PSP) is caused by protein phosphorylation through kinases such as Ca^{2+}-calmodulin-dependent protein kinase II (CaMKII; Lledo et al., 1995). This triggers the insertion and modulation of α-amino-3-hydroxy-5-methyl-4-

isoxazolepropionic acid (AMPA) receptors into the postsynaptic membrane (Benke, Luthi, Isaac, & Collingridge, 1998; Collingridge et al., 2004; S. Q. Liu & Cull-Candy, 2000) and synthesis of protein during late LTP (L-LTP; Hernandez & Abel, 2008). Basically, LTD shows a molecular cascade as complex as LTP, but in reversed order, e.g., by AMPA receptor removal (Bear, 1995; Bear & Malenka, 1994; Benington & Frank, 2003). In addition, intracellular [Ca^{2+}] increases are smaller and longer lasting than for LTP (Benington & Frank, 2003).

Synaptic efficacy describes "the capacity of a presynaptic input to influence postsynaptic output" (Lopez, 2002). Functional plasticity refers to synaptic efficacy alteration, which is quantified by changes in signaling amplitude (Munno, Prince, & Syed, 2003). These activity-dependent changes further induce structural modifications (Knott, Holtmaat, Wilbrecht, Welker, & Svoboda, 2006; Trachtenberg et al., 2002) that correlate with synaptic strength (Caroni, Donato, & Muller, 2012; Matsuzaki, Honkura, Ellis-Davies, & Kasai, 2004). Such long-term structural modifications (up to several weeks) include the formation and stabilization of synapses (Xu et al., 2009; Yang, Pan, & Gan, 2009) by alterations in gene expression and protein synthesis (Costa-Mattioli, Sossin, Klann, & Sonenberg, 2009). Structural plasticity is believed to be involved in long-term storage related to learning and memory (Bailey & Kandel, 2008).

So far, functional and structural changes were introduced as two separate processes. However, we have good evidence to belief that functional and structural changes may occur in parallel and are reversible (Holtmaat, Wilbrecht, Knott, Welker, & Svoboda, 2006; Knott et al., 2006). Thus, as summarized by Caroni et al., the time course of learning-induced LTP and the related structural plasticity is not well understood (Caroni et al., 2012). This means it is difficult to actually measure in which time range functional and/or structural plasticity occurs during learning processes.

In humans, direct measurement of synaptic plasticity is difficult. A possible approximation for assessing neuronal plasticity may be the application of intensive cognitive training which challenges plastic changes maximally. As one approach to investigate learning and learning-induced plastic changes, cognitive training may be used.

3.2.2. A behavioral measure for plasticity: investigating learning by using working memory training

In previous years, the interest in working memory training has grown. According to the early model of Baddeley and Hitch (Baddeley & Hitch, 1974), working memory serves for the temporary storage of information during cognitive processing and is related to higher cognitive functions such as reasoning and planning. Their multicomponent model consists of two slave systems, the phonological loop (verbal and acoustic information) and the visuospatial sketchpad, and an 'executive control' system, the attentional control (Baddeley, 2000; Baddeley & Hitch, 1974). Both slave

systems are limited in capacity, i.e. in the number of items that can be stored during information processing. During processing, attention is required to direct and assure the correct use of the stored items (Baddeley, 2000). Working memory is measured with test like n-back tasks (Kane, Conway, Miura, & Colflesh, 2007), which require both simultaneous processing and storage of information as well as attentional control. More recently, the effects of working memory were even shown to transfer to other cognitive tasks, e.g., to fluid intelligence (Jaeggi et al., 2008) or cognitive control (Chein & Morrison, 2010). Considering this aspect as well as the importance of working memory in a wide range of complex cognitive functions (e.g., reasoning, planning, reading comprehension, and problem solving; Unsworth, Redick, Heitz, Broadway, & Engle, 2009), studies which show that working memory may be trained (Klingberg et al., 2005; Olesen, Westerberg, & Klingberg, 2004; Thorell, Lindqvist, Bergman Nutley, Bohlin, & Klingberg, 2009; Westerberg & Klingberg, 2007) are of great interest. Assessing performance changes longitudinally (e.g., Dahlin, Dahlin, Nyberg, Bäckman, & Neely, 2008; Jaeggi et al., 2008; Jaeggi, Buschkuehl, Jonides, & Shah, 2011) offers one possibility to determine the effectiveness of working memory training, because sustained performance increases may be indicative for plastic changes, and not mere retest or practice effects (Freund & Holling, 2011; Hausknecht, Halpert, Di Paolo, & Moriarty Gerrard, 2007).

In order to determine the effectiveness of working memory training, other indicators than behavioral ones are additionally needed. Evidence for working memory training-induced plasticity was provided for example by an fMRI study conducted by Olesen et al. (Olesen et al., 2004). In this study, visuospatial performance increased gradually during five weeks of training and brain activity increased in prefrontal and parietal regions. The activity increase found after several weeks of training was suggested to represent skill acquisition related to cortical plasticity.

In conclusion, learning and synaptic plasticity may be investigated in humans on the basis of behavioral measures, as exemplified by the intensive working memory training. Yet literature on neurophysiological measures in relation to intensive cognitive learning is sparse. In the next section, sleep and its implication in learning and plasticity are introduced.

3.2.3. Sleep SWA and plasticity

An increasing number of studies indicate that plastic changes during wake and due to learning are reflected by changes in sleep slow waves, which is the marker for sleep homeostasis. Measuring multi-unit activity in rat cortical cells, Vyazovskiy et al. showed that neuronal activity during NREM sleep changes in response to sleep need (Vyazovskiy et al., 2009): at the beginning of a NREM sleep episode after prolonged wake, firing during the ON states was highly synchronous and the firing rate was higher than during ON states that were preceded by sleep. In addition, the changes in

firing rate during the ON states from early to late sleep correlated positively with the changes in NREM sleep SWA. Thus, these changes in neuronal firing from NREM slow wave sleep to wake are related to process S, and recent studies show that they reflect changes in synaptic strength. For example, slow waves during NREM sleep have been associated with net synaptic depression whereas the tonic firing rate during spontaneous wake is associated with net synaptic potentiation (Vyazovskiy et al., 2008). Molecular markers for synaptic potentiation such as glutamate receptor 1 (GluR1) -containing AMPA receptor levels as well as phosphorylation of AMPA and CaMKII were higher after wakefulness than after sleep. Thus, during wake, the trafficking of AMPA receptors and phosphorylation, both processes associated with LTP, seem to dominate whereas during sleep, mainly LTD is observed. These indicators for LTP and LTD were accompanied by electrophysiological correlates of synaptic strength: slope steepness of the response to cortical excitation increased with prolonged wakefulness and decreased during NREM sleep (Vyazovskiy et al., 2008). Other studies report on plasticity-related genes that are up-regulated during wake. For example the concentration of the brain-derived neurotrophic factor (BDNF), a marker for synaptic potentiation, is higher during wake than sleep (Cirelli & Tononi, 2000). Also, a BDNF increase after explorative behavior in rats is associated with increased SWA (Huber, Tononi, & Cirelli, 2007). Thus, sleep-wake dependent plastic changes may be observed after several hours, e.g., overnight. These changes may rely on the functional modification of synaptic strength (e.g., Z. W. Liu, Faraguna, Cirelli, Tononi, & Gao, 2010; Vyazovskiy et al., 2008). On a larger time scale, e.g., during development, also structural changes (synaptogenesis and pruning) were shown to be modulated by wake and sleep (Maret, Faraguna, Nelson, Cirelli, & Tononi, 2011). In adolescent mice, wake was associated with synaptogenesis, since net spine growth occurred during wake; sleep on the other hand was dominated by pruning, indicated by net spine loss (Maret et al., 2011).

The link between slow waves and synaptic strength was established in computer models (Esser et al., 2007), animal models (Vyazovskiy et al., 2007), and human subjects (Riedner et al., 2007). In these studies, slow wave slope and number of slow waves with high amplitude decreased from early to late sleep, reflecting decreased synchronization and thus, a decrease in strength of corticocortical connections. In cortical slices of mice and rats, also frequency and amplitude of miniature excitatory postsynaptic current (mEPSC), a direct measure of synaptic strength, were higher during wake than sleep (Z. W. Liu et al., 2010).

Those molecular and electrophysiological links to sleep need are supported by structural modifications. In drosophila, synapse size and number decreases from wake to sleep (Bushey, Tononi, & Cirelli, 2011). When inducing enriched experience, synapses grow even further and sleep need is increased. The observed morphological changes during wake were renormalized at subsequent sleep which was not the case for flies that were sleep deprived. This implies that sleep slow waves not only reflect,

but also contribute to synaptic plasticity. This notion is the basic statement of the synaptic homeostasis hypothesis.

Synaptic homeostasis hypothesis

It has been claimed that synaptic potentiation may not be extended infinitely but needs to be regulated in order to prevent saturation of network plasticity (Turrigiano, 1999). The synaptic homeostasis hypothesis states that sleep is the key mechanism to globally counterbalance the excessive synaptic plastic changes occurring during wake (Tononi & Cirelli, 2006). As shown by Vyazovskiy et al., wake is indeed associated with net synaptic potentiation (Vyazovskiy et al., 2008). But the steady increase in synaptic strength during wake due to synaptic potentiation is expensive concerning energy and space consumption. As proposed in a computer model by Olcese et al. (Olcese, Esser, & Tononi, 2010), synaptic renormalization during sleep may be a key for achieving a decrease in synaptic strength. This idea is supported by the finding that synaptic renormalization in drosophila is only observed in animals that were not sleep deprived (Bushey et al., 2011). On systemic level, correlates of energy expenditure and metabolic rate such as cerebral oxygen utilization were found to be higher in the evening compared to the morning (Braun et al., 1997). The higher metabolic rate may reflect the high synaptic strength at the end of a wake period. This aspect will be discussed in section 4.4, where hemodynamic changes over night as measured by NIRS will be presented.

According to the synaptic homeostasis hypothesis, the sequence of depolarized and hyperpolarized phases constituting the slow waves is involved in globally decreasing synaptic strength (downscaling). This would serve to overcome the expensive increase in synaptic strength. Downscaling refers to the proportional decrease of synaptic strength during sleep, which increases the signal-to-noise ratio. This process would allow strengthening of established connections or the acquisition of new information during the following wake episode.

The hypothesis is used to explain the link between sleep homeostasis and synaptic plasticity. As there is increasing support that slow waves are regulated locally and learning is localized to specific regions, changes in neuronal activity due to synaptic plasticity changes may be mapped by SWA.

Local slow wave activity

The local occurrence of slow waves has been shown in animals as well as in humans. For example in sleep deprived rats, single cortical neurons went transiently ‘offline’, showing OFF periods during wake comparable to NREM sleep (Vyazovskiy et al., 2011). In humans, intracortically measured activity of neuronal populations revealed that most sleep slow waves occurred locally (Nir et al., 2011). On the scalp, regional slow waves may be recorded and topographically mapped (as an example, see Fig.

3.3) by using high-density EEG (HD EEG), featuring up to 256 electrodes (for review, see Lustenberger & Huber, 2012).

Regarding the relationship between synaptic strength and slow waves described above, SWA may reflect the extensive use (or inhibition) of certain brain regions during the day and its underlying changes in synaptic strength. Early studies showed regional changes in NREM sleep in response to sleep deprivation (e.g., Finelli, Borbely, & Achermann, 2001). For example after prolonged wake, sleep SWA is highest in frontal electrodes (Cajochen, Foy, & Dijk, 1999). This may be due to the fact that frontal regions are highly active in the adult brain, e.g., for higher cognitive processes such as executive functioning (Smith & Jonides, 1999).

Manipulating synaptic plasticity seems to change SWA in subsequent sleep (Lustenberger & Huber, 2012), e.g., by modulating synaptic potentiation with transcranial magnetic stimulation (TMS). Applying TMS to the median nerve changed cortical excitability over sensorimotor cortex and consequently altered SWA in said region (Huber et al., 2008). Learning paradigms also led to local changes in SWA associated to synaptic plasticity. Learning a visuomotor adaptation task in the morning (Maatta et al., 2010) or evening (Huber et al., 2004) increased SWA in right parietal electrodes (see Fig. 3.3), a region that is linked to the task. Such learning-induced changes were also observed in a clinical population: In patients having suffered from stroke, speech and language therapy led to performance improvements that were reflected in local SWA changes (Sarasso et al., 2014). In consequence to these studies investigating synaptic potentiation, it was shown that a decrease in SWA may be indicative for synaptic depotentiation (Huber et al., 2006). Huber et al. showed that in humans, arm immobilization led to synaptic depression, which was shown by a decrease in SWA over the contralateral sensorimotor cortex in the subsequent sleep episode (Huber et al., 2006).

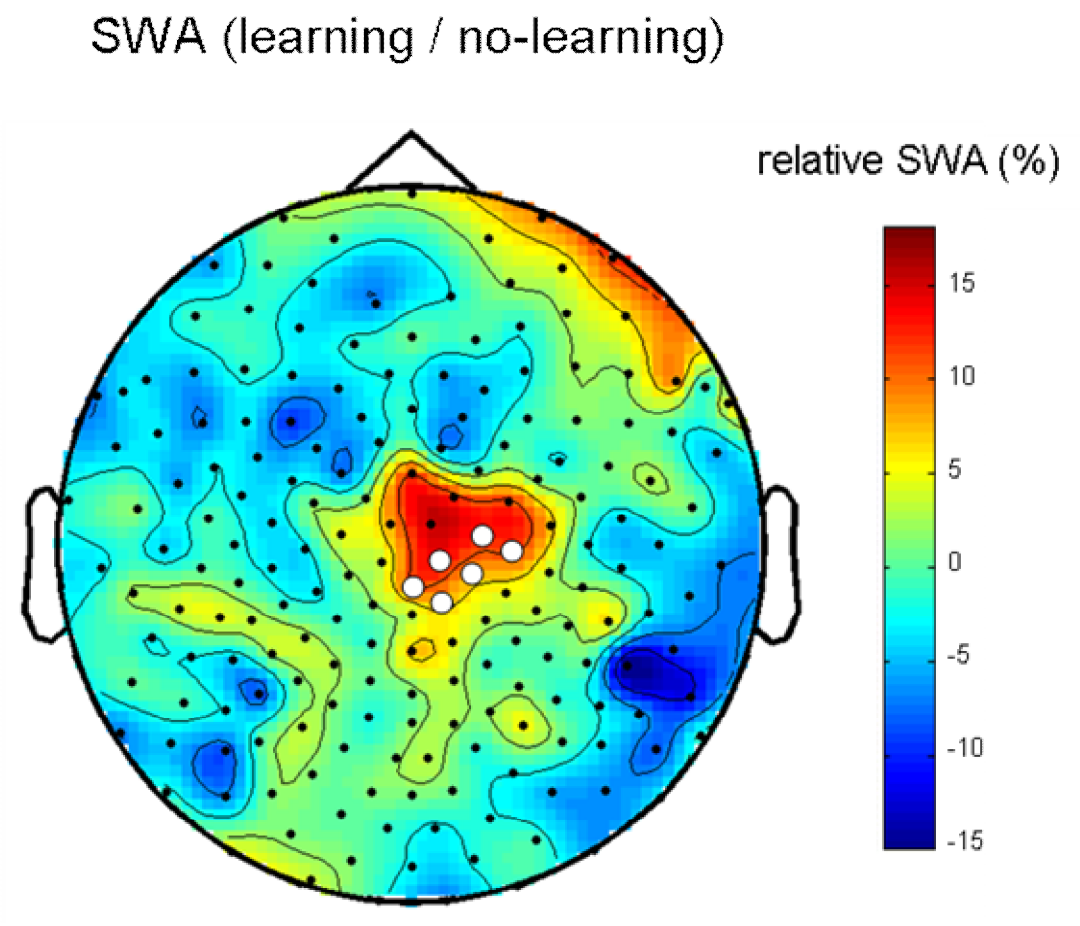

Figure 3.3: Local change in sleep slow wave activity (SWA) after a visuomotor learning task in adult subjects (adapted from Huber et al., 2004). The topographic plot shows SWA after learning (learning condition) in percent of baseline (no-learning condition); red indicates a relative increase and blue a relative decrease. The white dots indicate the electrodes where sleep SWA was significantly increased in the learning compared to the no-learning condition.

Regional changes in SWA were also observed over long time periods. From childhood to late adolescence, maximal SWA shifts from more occipital to frontal regions (Buchmann, Kurth et al., 2011; Kurth et al., 2010; Shaw et al., 2008). This finding is in line with reports on structural cortical maturation measured by magnetic resonance imaging (MRI; Buchmann, Kurth et al., 2011; Shaw et al., 2008). Peak cortical thickness is reached at late childhood and shifts from occipital to frontal regions from childhood to late adolescence (Shaw et al., 2008). The temporal pattern of brain maturation is paralleled by the development of cognitive functions (Gogtay et al., 2004). Cognitive functions such as working memory reach mature level during late adolescence (Luna, Garver, Urban, Lazar, & Sweeney, 2004). Accordingly, frontal areas, which are involved in higher cognitive functions such as executive functions (Smith & Jonides, 1999), mature later than for example the visual cortex (Gogtay et al., 2004; Shaw et al., 2006). The relationship between gray matter maturation and SWA was established by an MRI study, which showed that markers for cortical maturation (e.g., gray matter volume) correlated positively with SWA (Buchmann, Ringli et al., 2011). In addition, as recently reported by Kurth et al. (Kurth et al., 2012), SWA may

be used as a marker to distinguish skill maturation, which strengthens the parallel maturational processes observed in cognition, brain structure and SWA.

3.3. Goal, aims and hypotheses of the study

The goal of this project presented here was to examine neuronal activity during sleep by using EEG and NIRS and to investigate effects of cognitive training on sleep in relation to cognitive performance. Metabolic changes in relation to neuronal activity during sleep were investigated by introducing NIRS in combination with the established sleep HD EEG. NIRS serves as a tool to measure hemodynamic correlates of brain metabolism that accompanies neuronal activity. Furthermore, we were interested in the effects of intensive working memory training on cognitive performance. Considering the relationship between SWA and learning, we intended to use HD EEG (128 electrodes) to map learning-induced changes in SWA. Below, four hypotheses are formulated which constitute the main questions of this work.

We tested our hypotheses in young subjects aged between 10 and 16 years. At this age, sleep depth is high (Carskadon & Dement, 2011) which is also reflected by a peak in SWA at around prepuberty (Feinberg et al., 2006). As mentioned in the introduction, at this age, major plastic changes occur (Giedd et al., 1999; Huttenlocher, 1979) that parallel these changes in SWA. Thus, we expected the manipulation of synaptic plasticity to be most effective within a young population.

Hypothesis I *Complementary to EEG, NIRS serves as a tool to discriminate vigilance states.*

Firing rate exhibits distinct changes between wake and NREM sleep (Vyazovskiy et al., 2009). As neuronal activity differs between vigilance states, also energy demand of the brain and thus, blood supply is altered (e.g., Braun et al., 1997). One possibility to assess metabolic processes and monitor cerebral hemodynamics, as a surrogate for metabolic processes during sleep, is provided by NIRS (Uchida-Ota et al., 2008). NIRS offers a non-invasive approach (contrary to PET) and it allows investigating sleep over several hours without excessively confining the subject. Our aim was to measure transitions between vigilance states by applying a NIRS sensor into HD EEG in young subjects. We expected to be able to observe changes in hemodynamics synchronously to vigilance states as assessed by the EEG signal. Novel to our approach is the measurement of tissue oxygen saturation (StO_2) over an entire night. Only one study featuring a nap protocol has so far investigated StO_2 during sleep (Kubota et al., 2011). StO_2, calculated according to the self-calibrating principle (Hueber, Fantini, Cerussi, & Barbieri, 1999), is a reliable NIRS measure for cerebral perfusion in addition to the measurement of changes of [O_2Hb], [HHb] and [tHb]

over time (see section 4.1). To do so, we used a wireless in-house built NIRS sensor that was concurrently applied with EEG, which detects SWA.

Hypothesis II *Working memory training over three weeks is a measure for learning ability and improves cognitive skills short- and long-term.*

Working memory is an important function for cognitive reasoning and learning (Kane, Hambrick, & Conway, 2005). Having this in mind, we hypothesized that working memory training may serve as an appropriate tool to investigate learning capabilities and to induce cognitive plasticity. We further hypothesized that if learning, assessed by working memory training, induces relevant changes in cognition, then the effects of the training should sustain long-term. Therefore, we assessed long-term effects (2 to 5 months after the training) on cognitive performance with the aim to find an indicator for the effectiveness of the training.

We further considered that working memory training in adults was shown to exhibit transfer effects to fluid intelligence (Jaeggi et al., 2008). Jaeggi et al. claimed that transfer effects on fluid intelligence are possible because it is functionally linked to, and shares similar neural networks with, working memory (Jaeggi, Studer-Luethi et al., 2011). Also other cognitive functions, such as controlled attention (Baddeley, 2003) and processing speed (Fry & Hale, 1996), have been linked to working memory performance. Regarding these aspects, we hypothesized that working memory training may be beneficial for untrained cognitive tasks. We measured performance changes in working memory, fluid intelligence, attention, short-term memory and processing speed immediately and some months after working memory training (see section 4.2).

Hypothesis III *Learning-induced effects of working memory training lead to local SWA changes.*

As shown in animals (Vyazovskiy et al., 2008), changing synaptic connections by potentiation increases synchrony and amplitude of slow waves during sleep. Recent studies showed that changes in SWA serve as tool to map learning-induced plastic changes. In adult humans, Huber et al. found a significant increase in SWA after subjects had trained a visuomotor learning task (Huber et al., 2004). This local SWA increase correlated positively with overnight improvement in the performed task.

We assumed that the intensive working memory training over three weeks (see *Hypothesis II*) would lead to synaptic plastic changes, consequently altering SWA locally. For the first time, we assessed effects of learning on sleep SWA over a longer period than just over one night. Sleep SWA topography was examined with HD EEG in an overnight sleep session before and after three weeks of working memory training.

We hypothesized that the local changes in SWA would be associated with performance increases due to working memory training (see section 4.3).

Hypothesis IV *Complementary to EEG, NIRS serves as a tool to assess metabolic changes in the course of NREM sleep in young subjects.*

Not only vigilance states differ in their activity pattern but also regulatory aspects of sleep are known to reflect changes in firing rate. Thus, we addressed sleep regulation in relation to oxygen metabolism. As described in the introduction, the alternation between wake and sleep is homeostatically regulated (Achermann & Borbély, 2011). This homeostatic process is reflected by SWA during NREM sleep since SWA is highest after a period of wakefulness, when sleep need is high, and decreases during subsequent sleep. According to the synaptic homeostasis hypothesis (Tononi & Cirelli, 2006), SWA not only reflects this homeostasis between sleep and wake, but also plays a functional role in sleep regulation and plasticity. During wake, synaptic strength increases due to potentiation. In the following sleep episode, this process is balanced out by net synaptic depression (Vyazovskiy et al., 2008). Thus, according to the synaptic homeostasis hypothesis, slow waves may actively downscale the increased synaptic strength during wakefulness. These processes may lead to changes in metabolic turnover (see section 4.4). Thus, we were interested in the relation between sleep regulatory processes reflected by SWA and its relation to energy consumption and metabolic turnover overnight. Assessing the same variables as described in *Hypothesis I*, we hypothesized that changes in brain metabolism overnight may be observed as changes in StO_2.

4. Articles

4.1. Changes of cerebral tissue oxygen saturation at sleep transitions in adolescents

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Author contributions RH, PA and MW conceived the study. Together with AM and FP, they designed the study. Recruitment of participants and data assessment were done by AM and FP. Sleep data was processed by FP. Analysis was performed by AM. AM and MW wrote the first draft of the manuscript. All authors were involved in the revision of the draft manuscript and have agreed to the final content.

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Abstract In adults, cerebral oxy- ([O_2Hb]) and deoxyhemoglobin concentrations ([HHb]) change characteristically at transitions of sleep stages. The aims were to assess these changes in adolescents and additionally to measure tissue oxygen saturation (StO_2) by near-infrared spectroscopy (NIRS). Previously it was reported that in adults [O_2Hb] increased and [HHb] decreased at the transition from non-rapid eye movement sleep (NREMS) to REMS and wakefulness. Transitions to NREMS from REMS/wakefulness led to a decrease in [O_2Hb] and an increase in [HHb]. We measured [O_2Hb], [HHb] and tissue oxygenation (StO_2) with NIRS approximately above the left prefrontal cortex in 12 healthy adolescent males (aged 10 to 16 years). We found comparable signs and magnitudes of changes in [O_2Hb] and [HHb] as observed in adults. StO_2 increased at the transitions from NREMS to REMS and decreased from REMS to NREMS and at sleep onset (all $p < 0.01$, linear mixed effects model). Changes in oxygen metabolism during sleep transitions are similar in adolescents and adults. In addition, we show for the first time temporal changes of StO_2 at sleep transitions.

4.1.1. Introduction

Brain activity during sleep was widely studied with electroencephalography (EEG) so far. Another measure of brain activity is the cerebral oxygen metabolism (Wolf et al., 2008), even though the link is not completely understood. The cerebral blood flow (CBF, as a marker of oxygen metabolism and neuronal activity (Maquet, 2000) was assessed by positron emission tomography (PET) (Dang-Vu et al., 2010; Maquet, 2000). These data indicate that absolute global CBF is lower in non-rapid eye movement sleep (NREMS) compared to wakefulness and higher in rapid eye movement sleep (REMS) compared to NREMS. However, regional CBF changes were heterogeneous in the frontal (and parietal) areas during REMS (Dang-Vu et al., 2010; Maquet, 2000). Near-infrared spectroscopy (NIRS) non-invasively measures oxy- (O_2Hb), deoxyhemoglobin (HHb) and tissue oxygen saturation (StO_2) and therefore provides additional information of cerebral oxygenation compared to PET, but without spatial resolution. Several NIRS studies reported consistently an increase of [O_2Hb] and a decrease in [HHb] at the transition from sleep to wakefulness and opposite changes at transitions from wakefulness to sleep (Hoshi, Mizukami, & Tamura, 1994; Kubota et al., 2011; Näsi et al., 2011; Shiotsuka et al., 1998; Spielman et al., 2000; Uchida-Ota et al., 2008). These studies measured hemodynamic changes approximately in the left or right (or both) prefrontal cortex. At the transition from NREMS to REMS an increase in [O_2Hb] and a decrease in [HHb] was found (Kubota et al., 2011; Näsi et al., 2011) and at the transition from REMS to NREMS opposite changes were observed (Näsi et al., 2011). To our knowledge, only in one study (Kubota et al., 2011) StO_2 was measured in which they reported a decrease in StO_2 in NREMS compared to wakefulness and increased levels in REMS compared to NREMS. It should be noted that this study (Kubota et al., 2011) and in (Spielman et al., 2000) sleep was investigated only during an afternoon nap protocol. In all studies data were recorded in adults. We aimed to examine for the first time cerebral oxygenation in adolescents and StO_2 changes at sleep state transitions.

4.1.2. Methods

Subjects

We analyzed overnight sleep recordings of 12 healthy adolescent right- handed males (age 10 - 16 years, mean 12.6 years). Each participant spent two nights in the sleep laboratory of the University Children's Hospital Zurich, separated by 3 weeks. One recording was excluded due to technical difficulties. Twelve subjects with a total of 23 recordings were analyzed. The study was approved by the local ethical committee and informed consent was obtained from the legal representatives. For the detailed protocol see (Metz, Pugin, Huber, Achermann, & Wolf, 2013).

NIRS measurement

OxyPrem, an in-house built continuous wave NIRS device, electronically similar to a previous device (Muehlemann, Haensse, & Wolf, 2008), measures light attenuation at three wavelengths (760 nm, 805 nm and 870 nm) and at two distances (1.5 cm and 2.5 cm) with a sampling rate of 35 Hz. OxyPrem measures StO_2, [O_2Hb], [HHb] and total hemoglobin concentration ([tHb]) for two different areas using a self-calibrating approach (Hueber et al., 1999). Both regions cover an area of ~3 cm^2, one region being closer to electrode F3 (just below the hairline of the subject) and one closer to Fp1. The NIRS sensor was placed on the left forehead close to the electrode position Fp1 (international 10/20 system). NIRS and high-density EEG with 128 electrodes were recorded simultaneously for the entire night. An accelerometer (ADXL330, Analog Devices) in the NIRS sensor also registered the subject's movements. Data were similar in both regions and we therefore only report results of the F3 region.

Post-processing

We calculated StO_2 by the diffusion approximation without accounting for water in the tissue, as described in assumption A4 in (Metz, Biallas, Jenny, Muehlemann, & Wolf, 2013). The algorithm is described in detail in (Metz, Pugin et al., 2013). [HHb] and [O_2Hb] were corrected for movement artefacts based on (Scholkmann, Spichtig, Muehlemann, & Wolf, 2010; Virtanen, Noponen, Kotilahti, & Ilmoniemi, 2011). This algorithm adapts the level after an artefact to the level before the artefact if the difference between the two levels exceeds the 95 % confidence interval of the signal (within a chosen time frame of 20 s according to (Virtanen et al., 2011) prior to the artefact. Additionally, the level was adjusted to the one in periods where the signal is assumed to be correct based on the accelerometer data. From the corrected [HHb] and [O_2Hb], StO_2 was calculated as [O_2Hb] / ([O_2Hb] + [HHb]) and [tHb] as [O_2Hb] + [HHb].

Sleep stages were scored according to standard criteria (Iber et al., 2007). We focused on the following transitions: NREMS to REMS, REMS to NREMS (based on NREM-REM sleep cycles (Feinberg & Floyd, 1979), and sleep onset, which was defined as the first appearance of stage N2 (NREMS is divided into stages N1-N3, N1 being lighter sleep and N3 deep sleep). Time 0 was set at each transition and defined as center of a window of 10 min (i.e. ± 5 min). We ensured that these 5 min before or after the transition contained at least 75 % of the respective states. Prior to statistical testing the signals were first low pass filtered at 0.2 Hz. Second, the mean of each 10 min window was subtracted for each time-point within the window. Third, median time courses were calculated over multiple appearances of one transition within a night, then over the two nights per subject and finally over the 12 subjects. As measure of variability, the median absolute deviation (MAD) was determined (Fig. 4.1.1 and Table 4.1.1). For display reasons the median and the MAD were smoothed by a

moving average of 1 min (Fig. 4.1.1). Data processing was performed in Matlab® (R2011b, The Mathworks®, Natick, MA, USA).

Statistics

The first and the last minute of the 10 min window were compared to test for significant changes at the transitions. To account for two nights per subject and multiple transitions per night we applied a linear mixed effects model (function "lme" in R version 2.15.2, R Core Team, Vienna, Austria) with the fixed effects "time" (last - first minute), "night" (different nights) and "transition #" (multiple transitions within a night) and subject as random effect. To account for multiple testing (four different signals - StO_2, HHb, tHb and O_2Hb, two different regions and three different transitions) we applied the false discovery rate (FDR) correction at the 5 % significance level (Benjamini & Hochberg, 1995).

4.1.3. Results

StO_2, [tHb] and [O_2Hb] increased at the transition from NREMS to REMS and StO_2 decreased during the transition from REMS to NREMS. In contrast, [HHb] increased at the transitions from REMS to NREMS and at sleep onset (Table 4.1.1; Fig. 4.1.1). In total 77 NREMS to REMS, 62 REMS to NREMS transitions and 21 sleep onset periods were analyzed. At the transition from NREMS to REMS the increase in StO_2 (and [tHb], [O_2Hb]) started 72 s before the actual transition. StO_2 gradually decreased and [HHb] increased at the transition into sleep, i.e. the change started several minutes prior to sleep onset (defined as first occurrence of N2; Fig. 4.1.1). The changes at any of the transitions were similar in the two measured regions (data not shown).

Transition	Δ[O_2Hb] ± MAD [μM]	Δ[HHb] ± MAD [μM]	ΔStO_2 ± MAD [%]	Δ [tHb] ± MAD [μM]
NREMS to REMS	+ 1.80 ± 1.41 *	- 0.22 ± 0.40	+ 0.67 ± 0.47 *	+ 1.32 ± 1.36 *
REMS to NREMS	- 0.65 ± 0.62	+ 0.45± 0.39 *	- 0.38 ± 0.32 *	- 0.17 ± 0.77
Sleep onset	- 1.12 ± 1.95	+ 0.81 ± 0.91 *	- 0.89 ± 1.13 *	+ 0.25 ± 2.1

Table 4.1.1. The median difference in [O_2Hb], [HHb], StO_2 and [tHb] between the last and the first minute of the 10-minute window at transitions are reported. Asterisks indicate a significant change (linear mixed effects model and false discovery rate correction, corrected significance level of 0.0278). MAD indicates the median absolute deviation of the difference, using Gaussian error propagation. StO_2 changes are provided in absolute per cent.

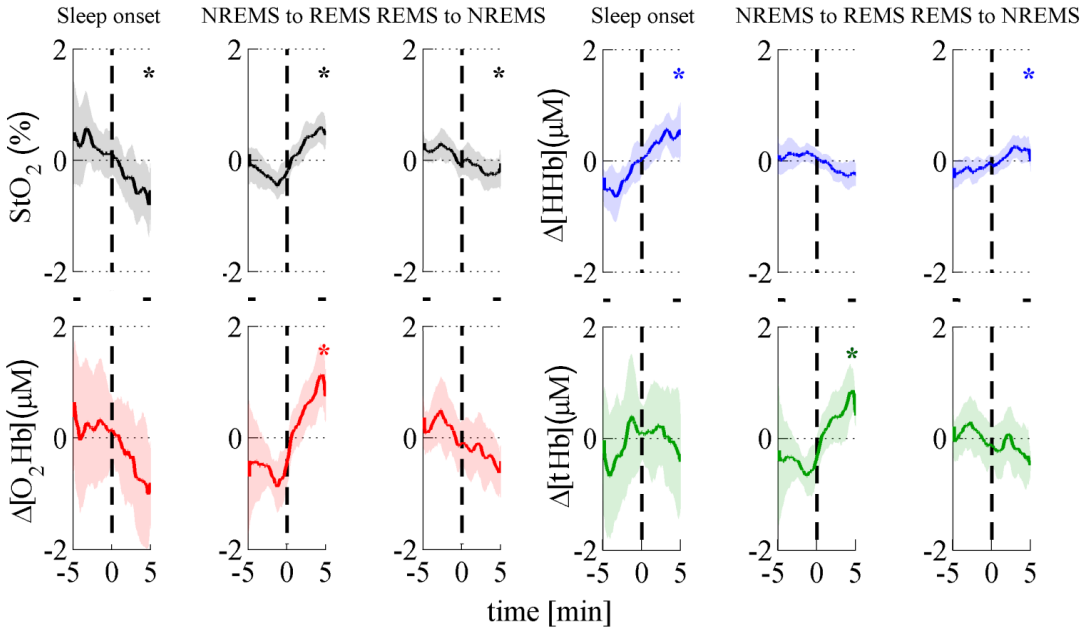

Figure 4.1.1. Median time course at sleep onset and sleep state transitions for tissue oxygen saturation (StO_2, black), oxyhemoglobin ([O_2Hb], red), deoxyhemoglobin ([HHb], blue) and total hemoglobin ([tHb], green) in region F3. The mean of the 10 min window was subtracted from each time course. The shaded area depicts the median absolute deviation. Time zero represents the transition (sleep onset, first epoch of NREMS or REMS). The horizontal black bars between the panels delineate the data that were compared by statistical testing. Data were low pass filtered by a 1 min moving average for display reasons. NREMS: non-rapid eye movement sleep. REMS: rapid eye movement sleep. Stars mark a significant change after false discovery rate (FDR) correction. Twelve subjects contributed two nights except one and multiple transitions per night to each transition (except for sleep onset).

4.1.4. Discussion and conclusion

Our results indicate that the change in the cerebral hemodynamics and tissue oxygenation in adolescents at the sleep state transitions NREMS to REMS, REMS to NREMS and wakefulness to NREMS is similar in sign and magnitude to those reported in adults (Hoshi et al., 1994; Kubota et al., 2011; Näsi et al., 2011; Shiotsuka et al., 1998; Spielman et al., 2000; Uchida-Ota et al., 2008). For the first time we showed the temporal change of cerebral StO_2 at these transitions. Although Kubota et al. (Kubota et al., 2011) presented StO_2 values for different sleep stages during a nap protocol, they did not show the temporal change at transitions. Additionally, we found similar changes at the transition NREMS to wakefulness (i.e. arousal) as (Näsi et al., 2011), but we excluded this transition from our analysis due to their small number of occurrence. PET literature reports that the global CBF is lower during NREMS compared to wakefulness and is elevated again during REMS to approximately the same level as in wakefulness (Braun et al., 1997; Maquet, 2000). These findings point in the same direction as the NIRS findings where we also see an increase in [tHb] at transitions into REMS. [tHb] represents CBV and is related to the CBF (Grubb, Raichle, Eichling, & Ter-Pogossian, 1974). For parts of prefrontal cortices, PET data indicate a reduction (Maquet, 2000) of relative regional CBF in

REMS compared to NREMS and thus, of cortical activity; for other parts no activation or an increase was observed (Maquet et al., 2005). This may be misleading since the statistical contrast is often based on a mixture of NREMS and wakefulness or solely wakefulness whereas our findings are reflecting state transitions directly. The relative CBF change in the prefrontal cortices seen with PET may also be masked by the global change in CBF. Our findings (and the global changes found with PET) indicate a cerebral activation during REMS and a cerebral deactivation at transitions into NREMS in the left prefrontal cortices. This interpretation is based on the assumption that the link between neuronal activity, oxygen consumption, CBF and the hemoglobin parameters (neurovascular coupling (Wolf et al., 2008) hold also during sleep. The decreased StO_2 during NREMS may be related to the reduced spiking activity in NREMS compared to wakefulness and REMS found in rats (Vyazovskiy et al., 2009). Spiking activity being responsible for ~ 47 % of total grey matter energy expenditure (in rodents) (Attwell & Laughlin, 2001) and hence may play a cardinal role in the oxygen metabolism. Näsi et al. (Näsi et al., 2011) found that the [O_2Hb] and [HHb] changes at sleep transitions (measured at 4 cm distance) were correlated to [O_2Hb] and [HHb] changes in the scalp (measured at 1 cm distance) and to heart rate using a principle component analysis. They suggested that the "cortical hemodynamic changes must correlate with systemic hemodynamic changes" but "this does not show that there are no cortical changes associated with the transitions" (Näsi et al., 2011). To avoid influences from the scalp we used a multi-distance approach (Hueber et al., 1999), which removes such a bias. A possibly important variable is the CO_2, which depends on the sleep stage (Shore, Millman, Silage, Chung, & Pack, 1985) and may influence StO_2 (Scholkmann, Gerber, Wolf, & Wolf, 2012). In the future, CO_2 should be measured additionally.

In conclusion, with NIRS we were able to non-invasively show that changes in [O_2Hb], [HHb] and [tHb] in adolescents at sleep stage transitions are similar to those in adults and for the first time we demonstrated the time course of the cerebral StO_2 at sleep transitions.

4.2. Working memory training shows immediate and long-term effects on cognitive performance in children and adolescents

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Author contributions MW, OGJ and RH conceived the study. Together with FP and MS, they designed the study. Recruitment of participants and data assessment were done by FP, AM and MS. Analysis was performed by FP and MS. FP, OGJ and RH wrote the first draft of the manuscript. All authors were involved in the revision of the draft manuscript and have agreed to the final content.

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Under review

Abstract Working memory is important for mental reasoning and learning processes. Several studies in adults and school-age children have shown performance improvement in cognitive tests after working memory training. Our aim was to examine not only immediate but also long-term effects of intensive working memory training on cognitive performance tests in children and adolescents. Fourteen healthy male subjects between 10 and 16 years trained a visuospatial n-back task over 3 weeks (30 min daily), while 15 individuals of the same age range served as a passive control group. Significant differences in immediate (after 3 weeks of training) and long-term effects (after 2 - 6 months) in an auditory n-back task were observed compared to controls (2.5 fold immediate and 4.7 fold long-term increase in the training group compared to the controls). The improvement was more pronounced in subjects with an increase in training performance. Other cognitive functions (matrices test and Stroop task) did not change when comparing the training group to the control group. We conclude that spatial working memory training in children and adolescents boosts performance in similar memory tasks such as the auditory n-back task. The sustained performance improvement several months after the training supports the effectiveness of the training.

4.2.1. Introduction

Learning is crucial for adaptation to new situations and is essential for improvements in cognitive functions over time. One important aspect of learning-related improvements in cognitive functions is working memory, which is the ability of simultaneously store and process information held in mind despite irrelevant, potentially interfering stimuli (Engle, 2002). It is generally accepted that working memory processes support higher cognitive functions including reasoning (Engle, 2002). Several studies have assessed working memory capacity during childhood and around the age of school entry, at a time when the learning load is large. For example, Alloway et al. showed that working memory impairment seems to be disadvantageous for learning abilities in reading and mathematics (Alloway, Gathercole, Kirkwood, & Elliott, 2009). In addition, using the Conners' Teacher Rating Scale, school teachers have reported high inattentiveness and executive problems in those children with poor working memory (Alloway et al., 2009). Regarding the predictive power of working memory for learning processes, it is not surprising that difficulties in cognitive functions such as reading (Wang & Gathercole, 2013) and mathematics (Passolunghi & Mammarella, 2012) are associated with working memory skills. For example, Wang et al. showed that children between 8 and 10 years with difficulties in single word reading performed worse in simple and complex span tasks compared to controls, two widely used working memory tasks (Wang & Gathercole, 2013). In another study, mathematical learning disabilities of school-age children were shown to be associated with low performance in spatial working memory (Passolunghi & Mammarella, 2012). Considering the impact of working memory on a wide range of cognitive functions, the enhancement of working memory is of great interest. Studies in adults showed that working memory may be trained in elderly people of around 80 years (Buschkuehl et al., 2008) as well as in young adults in their twenties (Jaeggi et al., 2008). Also in pre-school children, visuospatial working memory training improved untrained verbal working memory performance (Thorell et al., 2009).

Some of these studies not only showed significant performance increases in the trained working memory tasks, but also in other, untrained working memory tasks (Buschkuehl et al., 2008) and functionally more distant tasks such as fluid intelligence tests (Jaeggi et al., 2008). In a more recent study involving school-age children of around 9 years, Jaeggi et al. also investigated the long-term effects of working memory training (Jaeggi, Buschkuehl et al., 2011). Immediately after the training, they observed significant higher fluid intelligence in the group with a large training gain compared to the small gain group or a control group. Thus, the larger the training improvement, the greater were the transfer effects. Interestingly, the gain in fluid intelligence remained stable after three months without additional training in the large training gain group (Jaeggi et al., 2008).

The aim of our study was to investigate working memory training and its effects on working memory tasks and fluid intelligence in male subjects between 10 and 16 years. In fact, this age range may be particular susceptible to interventions because many cognitive functions are still developing (Luna et al., 2004). Furthermore, working memory performance has been shown to be linked to attentional control (Baddeley, 2003) and processing speed (Fry & Hale, 1996). Thus, putative transfer effects on fluid intelligence may not be limited to fluid intelligence, but may also include other cognitive functions. Hence, in addition to working memory and fluid intelligence tasks, we also measured inhibition and interference tasks as well as standardized processing speed. Finally, long-term effects may be indicative for the effectiveness of the training, thus, cognitive testing was repeated not only immediately after the training period but also a few months later.

4.2.2. Material and methods

Subjects

The participants were recruited through print media and announcements (e.g., community centers, sport clubs, schools). Inclusion criteria (evaluated by phone screening questionnaires) were: male, age between 10 to 16 years, good general health, right-handedness, no neurological disorder or other disease, no learning disabilities, no smoking or heavy alcohol or caffeine consumption (more than one serving per week). Parents and their children gave written informed consent after explanation of the study methods and aims. In order to assign the subjects to the experimental and control group, we used a stratified randomization with age and cognitive performance in matrices test and letter-number-sequencing (MAT and LNS, see below) as stratification factor.

Procedure

We assessed the cognitive performance before (PRE) and after 3 weeks (POST) of training. In addition, all the subjects were asked to participate in a third session after a minimum of 2 months (FOLLOW-UP = FU, Fig. 4.2.1). In one subject (control subject, code 36), POST took place 1 week later due to sickness on the planned test date.

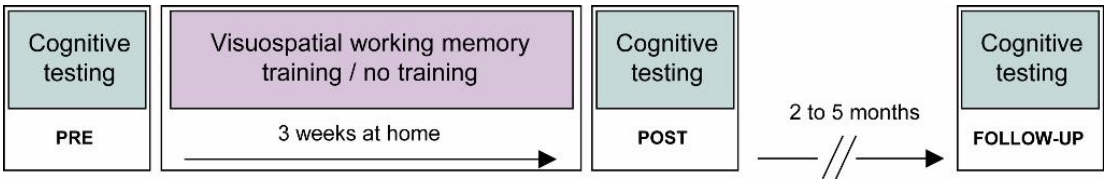

Figure 4.2.1: Scheme of the study procedure and training timeline. Cognitive testing included two working memory tasks (auditory n-back and letter-number sequencing), a fluid intelligence task [matrix reasoning task, TONI-IV (Test of Non-verbal Intelligence Version IV)], two cognitive control tasks (Stroop and Flanker task), two processing speed tasks (symbol search and digit-symbol substitution task) and a short-term memory task (number-span task). In addition, subjective motivation and concentration were measured on a scale from 1 (minimal) to 10 (maximal).

Within the 3 weeks between PRE and POST, 14 of the subjects completed an intensive working memory training programme (training group), which consisted of an adaptive visuospatial n-back task (Buschkuehl, Jaeggi, Kobel, & Perrig, 2007). The passive control group (N = 15) did not receive any working memory training.

The subjects were unaware of their group affiliation until the end of the first cognitive test session (PRE). The day after PRE testing, each subject from the training group was introduced to the training task by the research assistant. This included detailed information about the task and the setting as well as completion of a self-motivational control sheet. After this first supervised training session, participants were able to independently perform the training sessions at home. Thereafter, participants were asked to train for a maximum of 30 minutes per day over the following 3 weeks. Within these 20 days, they were visited once at home at an agreed date by a research assistant (not the cognitive test examiner). During this visit, the working memory training compliance was evaluated. For two subjects, the home visit did not take place due to organizational reasons (control group subjects code 15 and 28). Three weeks after PRE testing, the cognitive testing was repeated (POST) and some months later, the subjects participated in the third test session (FU, range 2 months 22 days to 5 months 6 days). No difference in the timing of FU was observed between the groups (training group: 3 months 21 days ± 6.44 mean ± SEM; control group: 3 months 16 days ± 4.77 mean ± SEM, unpaired t-test).

All participants received a present for taking part in the 3 cognitive test sessions. For participating in the 3 weeks of training, the members of the training group were given a small present of choice.

Setting

Cognitive performance of all but one subject was assessed by the same examiner. For one subject (code 28), the examiner was different at PRE due to organizational limitations. For each subject, the place of cognitive testing, time of day and week day were kept constant for all three sessions. Due to several reasons (e.g., time

		TRAINING GROUP			**CONTROL GROUP**								
Mean (SEM) Age [years]		12.97 (0.40)			13.23 (0.37)								
		PRE	**POST**	**FU**	**PRE**	**POST**	**FU**	**mixed-model ANOVA**					
Function	***Measure***	**Mean (SEM) [N]**	**Mean (SEM) [N]**	**Mean (SEM) [N]**	**Mean (SEM) [N]**	**Mean (SEM) [N]**	**Mean (SEM) [N]**	**[group x test session]**	η^2	**[test session]**	η^2	**[group]**	η^2
weeks after PRE		-	3	15.92 (0.89)	-	3 (one subject: 4)	15.26 (0.66)						
Working memory	*ANB*	3.46 (0.22) [13]	4.92 (0.37) [13]	5.17 (0.32) [12]	3.71 (0.37) [14]	4.29 (0.22) [14]	4.07 (0.19) [14]	* F(1,23) = 7.06 p = 0.003 [a]	0.235	F = 0.48 p = 0.60	0.020	(*) F = 3.93 p = 0.06	0.146
	LNS	11.86 (0.57) [14]	12.64 (0.85) [14]	12.14 (0.64) [14]	11.80 (1.07) [15]	11.93 (0.56) [15]	11.36 (0.44) [14]	F(1,25) = 0.19 p = 0.78	0.008	F = 0.37 p = 0.65	0.014	F= 0.31 p = 0.58	0.012
Fluid Intelligence	*MAT*	105.29 (3.35) [14]	111.29 (2.86) [14]	114.07 (2.93) [14]	109.80 (3.11) [15]	111.13 (2.86) [15]	110.64 (3.56) [14]	F(1,26) = 1.25 p = 0.30	0.05	(*) F = 2.93 p = 0.07	0.10	F = 0.0 p = 0.99	0.000
Inhibition	*ST (RT)*	37.87 (4.73) [12]	29.76 (3.37) [12]	22.56 (2.57) [13]	41.99 (5.35) [14]	33.16 (4.67) [14]	36.15 (6.29) [13]	(*) F(1,21) = 2.61 p = 0.09 [a]	0.11	F = 2.14 p = 0.14	0.09	F = 1.843 p = 0.19	0.081
Interference	*FT (RT)*	1531.88 (404.31) [12]	769.84 (265.74) [12]	816.81 (168.03) [12]	1441.62 (279.08) [15]	639.16 (104.77) [15]	564.85 (69.08) [14]	F(1,23) = 0.31 p = 0.65 [a]	0.01	* F = 4.96 p = 0.02	0.18	F = 0.15 p = 0.70	0.007
Processing Speed	*SST*	11.50 (0.66) [14]	12.93 (0.82) [14]	13.15 (0.94) [13]	11.67 (0.72) [15]	13.33 (0.77) [15]	13.43 (0.94) [14]	F(1,25) = 0.05 p = 0.95	0.002	** F = 12.08 p = 0.00	0.33	F = 0.13 p = 0.73	0.005
	DSS	10.93 (0.53) [14]	12.43 (0.76) [14]	12.21 (0.61) [14]	10.73 (0.83) [15]	12.53 (0.90) [15]	12.93 (0.95) [14]	F(1,26) = 1.35 p = 0.27	0.05	** F = 17.89 p = 0.00	0.41	F = 0.01 p = 0.91	0.000
Short-term memory	*NST*	6.08 (0.29) [13]	5.92 (0.37) [13]	6.31 (0.29) [13]	5.6 (0.31) [15]	6.07 (0.27) [15]	5.64 (0.25) [14]	(*) F(1,24) = 2.8 p = 0.08 [a]	0.105	F = 0.85 p = 0.43	0.034	F = 1.48 p = 0.24	0.058
Motivation	*Scale (1 to 10)*	8.17 (0.49) [12]	7.42 (0.60) [12]	6.67 (0.63) [12]	7.93 (0.34) [14]	7.14 (0.65) [15]	6.77 (0.49) [14]	F(1,23) = 0.18 p = 0.83	0.008	* F = 6.70 p = 0.003	0.23	F = 0.04 p = 0.85	0.002
Concentration	*Scale (1 to 10)*	7.50 (0.50) [12]	6.50 (0.61) [12]	6.00 (0.51) [12]	7.07 (0.51) [14]	6.57 (0.36) [15]	6.77 (0.59) [14]	F(1,23) = 1.03 p = 0.36	0.04	* F = 4.09 p = 0.03	0.15	F = 0.08 p = 0.78	0.004

Table 4.2.1: Mean ± SEM scores for cognitive tests, motivation and concentration. PRE = session 1; POST = session 2, three weeks after session 1; FU = follow-up after 15.59 ± 0.56 weeks (mean ± SEM) after POST. Statistics: mixed-model ANOVA with factors 'test session' (PRE, POST, FU) and 'group' (training, control) for each measure. * $p < 0.05$, (*) < 0.1; [a] = age as covariate. Effect size: η^2 square; RT = reaction time.

limitations or due to the participant's lack of motivation), the number of subjects was unequal for the cognitive tests (Table 4.2.1).

Intervention

Visual n-back training (VNBT, computerized version, BrainTwister software, (Buschkuehl et al., 2007))

One important aspect of working memory training is the adaptation of the difficulty level to the subject's performance. The working memory load should always be maximal (Jaeggi, Buschkuehl, Perrig, & Meier, 2010). For each stimulus in a series of consecutive trials, the participants had to remember the position of a blue square on a black computer screen and indicate by button-pressing when the square was in the same position as n before. During the test, the participants were supposed to fixate on a cross in the middle of the screen. Per trial, there were 8 possible positions of the square, randomized in every run. Over 20 days, the participants were requested to train for 30 minutes per day. Each of the training sessions started with n = 2 and included several series (trials per series: 20 + n). After each series, the feedback on performance in percent was displayed (only wrong trial clicks were included in the performance evaluation). The n increased if the performance level was over 90 % or decreased with a performance level of 70 % or less (Buschkuehl et al., 2007).

Cognitive testing

Auditory n-back task (ANB, computerized version, BrainTwister software)

The auditory n-back task (Buschkuehl et al., 2007) was based on the same underlying principle as the visuospatial training task, but with computerized spoken letters (C, I, K, Q, W, etc.) instead of visual squares. The duration of the task was restricted to 10 minutes. The maximal n of PRE, POST and FU were used as the dependent variable.

Letter-number sequencing task (LNS, German version of the WISC-IV)

The letter-number sequencing task was used as an additional task to assess working memory performance (Petermann & Petermann, 2007). In this task, the examiner orally presented three times the same length of span, including a mixture of letters and numbers. The subject had to remember the span and recite first the numbers in ascending order, then the letters in alphabetical order. As soon as the subject gave an incorrect answer for all three trials for a certain span, the task was finished. The number of the last correct span served as dependent variable (age standardized values).

Matrix reasoning task (MAT, Test of Nonverbal Intelligence (TONI IV))

As a measure of fluid intelligence, we used the matrix reasoning task TONI-IV (Brown, Sherbenou, & Johnsen, 1997) including two versions, from which the order (A, B, A or B, A, B) was balanced between the groups (age standardized values available). In the task, the participants had to choose the only pattern that completed the matrix presented from a given sample of patterns. The tasks stopped when three of five consecutive trials were not correctly solved or if the maximal trial number was reached (60, none of the subjects reached the maximum).

Stroop task (ST, paper version)

For a measure of inhibition, we employed a paper version of the Stroop task (one version and no age standardized values available) including three paradigms: 1) reading written color names, 2) naming the colors of lines and 3) naming color-words (incongruent paradigm), where the written color name is different from the ink color of the written word. The participants had to name the ink color of the word as fast as possible, thereby inhibiting to read out the written (semantic) color name. The duration (in seconds) and the errors per paradigm were assessed. As a measure of inhibition, the median time for the incongruent paradigm was used in our study (Bäumle, 1985).

Flanker task (FT, computerized version)

For a measure of stimulus-response interference we applied a computerized version of the Flanker task. The task was programmed with Presentation 14.8 (Neurobehavioral Systems) according to Stins et al. (Stins, Polderman, Boomsma, & de Geus, 2007). The participants had to indicate by button pressing (keyboard letter ‘a’ for left and ‘l’ for right) the pointing direction of the middle arrow of the randomly presented trials (condition neutral: < or >, condition congruent: <<<<< or >>>>>, condition incongruent: <<><< or >><>>). The difference between the reaction times for incongruent and congruent trials served as dependent variable.

Symbol search task and digit symbol substitution task (SST, DSS, German version of the WISC-IV)

Processing speed was assessed with symbol search and digit symbol substitution task (Petermann & Petermann, 2007). These tasks demand for quick and accurate responses, thereby challenging the ability of speed, accuracy and attention.

In each trial of the symbol search task, a target symbol was compared with a group of diverse symbols, and the participant indicated with YES or NO whether the chosen symbol was part of the presented group. In the second processing speed task, the DSS (Petermann & Petermann, 2007), the participant had to assign a series of numbers to simple geometric symbols, according to a given key. Both tasks had to be

solved as quickly as possible. The dependent variable was the number of correct trials within a certain time range (age standardized values).

Number-span task (NST, KAI)

The number-span task, assessed with the KAI (Lehrl, Gallwitz, Blaha, & Fischer, 1991), measures short-term memory. In this task, the participants had to repeat a span of numbers that was spoken by the examiner with a regular rhythm of one second. With every correct repetition, the length of the span increased, until the subject gave a false response, resulting in a following span with the same length. With a second error, the task was stopped. The maximally reached span length and the number of errors served as dependent variable (two versions, no age standardized values available).

Subjective motivation and concentration

Before each cognitive test session, the participant was asked to rate his motivation and concentration on a scale (*1 = not at all* to *10 = highly* motivated or concentrated).

Analysis and statistics

The training performance was calculated from the individual mean n (n indicates the level of n-back performance, see VNB and ANB above) of each training session. Each session started at n = 2, independent of performance on the previous session. Thus, we decided to consider the first three runs of each session as adaptation runs and excluded them from the analysis. For each training participant, their maximal performance and their respective session were determined over the entire training period. The difference between maximal performance and the last training session (last level) and the level at the first training session (start level) were used as a measure for training gain. The training amount was defined as the number of runs.

Statistical analysis was performed with SPSS software (PASW Statistics 18). The normal distribution of the cognitive variables was tested with Kolmogorov-Smirnoff test. The effects of the training on cognitive test performance were calculated with a mixed-model ANOVA, including factor 'group' (training, control) and factor 'test' (PRE, POST, FU), for each cognitive test. For tests with no age standardized values, age served as a covariate. Unpaired and paired t-tests were calculated for between-group respectively within-group effects. Due to the low number of participants, statistical trends were not considered.

4.2.3. Results

Working memory training performance changes

In a first step, we analyzed the visuospatial n-back (VNB) training amount and improvement by comparing performances at the first session with the performances at the last session and with the individual maximal performances (Fig. 4.4.2).

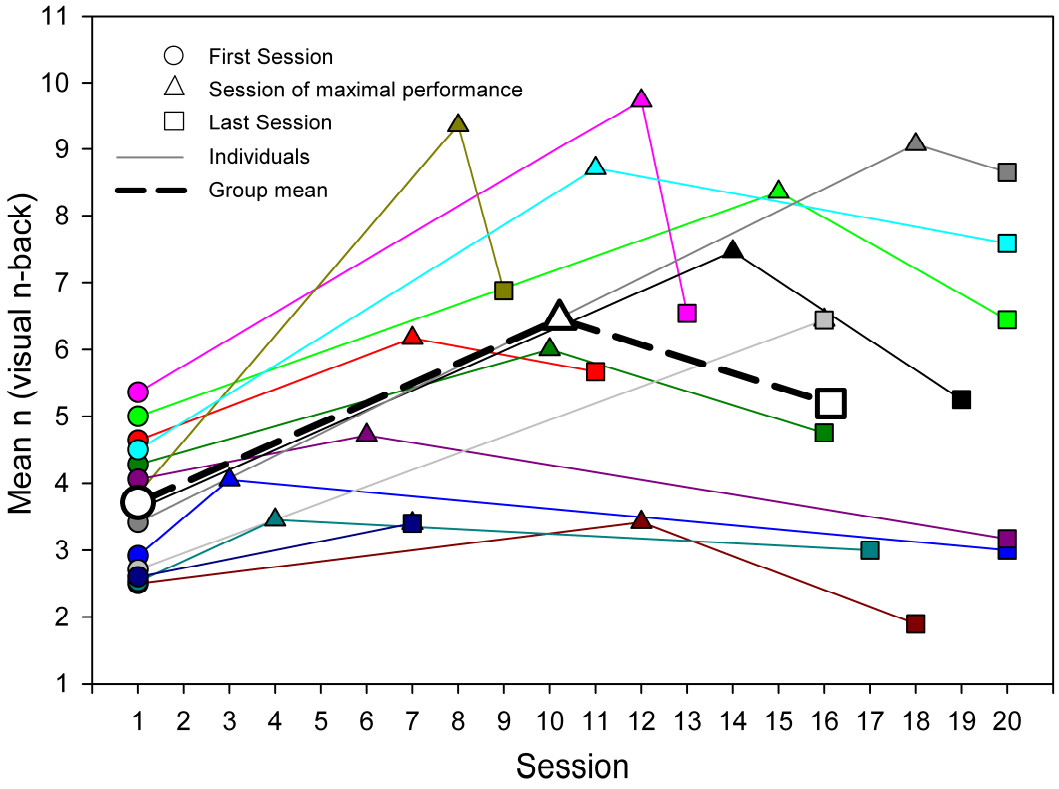

Figure 4.2.2: Individual training performance (first session, session of maximal performance, last session). Each solid line represents the performance of an individual (N = 14) in the visuospatial n-back (VNB) task training mean n of VBN at the first training session (circle), at the session of maximal performance (triangle) and at the last training session (square). The dashed line represents the average performance at the first session, the session of maximal performance and the performance at the last session. Average maximal performance was reached between session 10 and 11 (mean 10.21 ± SEM 1.22) and average performance at the last session was reached between session 16 and 17 (16.14 ± 1.19).

Participants trained on average for 16.1 ± 1.2 days (mean ± SEM, range: 7 to 20 days). Training performance was significantly increased from the first to the last training session (1.48 ± 0.46 mean ± SEM, range -0.89 to 5.24, paired t-test, $p < 0.05$). Maximal performance was reached on average between session 10 and 11, at 66.9 % ± 7.4 (mean ± SEM, Fig. 4.2.2) of the individual training time, and was significantly higher than performance at the end of the training (2.74 ± 0.49 mean ± SEM, range 0.66 to 5.65, paired t-test, $p < 0.001$, Fig. 4.2.2).

To assess the effect of age on training performance, we performed a correlational analysis. Performance during the first session was positively correlated with age

(Pearson correlation, r = 0.76, $p < 0.05$), that is the older the child, the higher the initial performance. Gain or amount of training, however, was not correlated with age. Initial performance also positively correlated with maximal performance during the training (partial correlation with age as covariate, r = 0.59, $p < 0.05$).

Effects of working memory training on cognitive performance

In a next step, we analyzed performance in each cognitive test at PRE, POST and FU, comparing the training with the control group (Table 4.2.1). A mixed-model ANOVA test revealed a significant difference between 'group' and 'test session' in auditory n-back (ANB) performance (Table 4.2.1). No other test (letter-number sequencing task, number-span task, matrix reasoning task, Stroop task, and Flanker task) showed a significant change. Between-group analysis of performance differences showed significantly higher increases in maximal ANB performance from PRE to POST and to FU in the training compared to controls (Fig. 4.2.3). The number of days between PRE and FU did not correlate with the improvements in ANB (Pearson correlation).

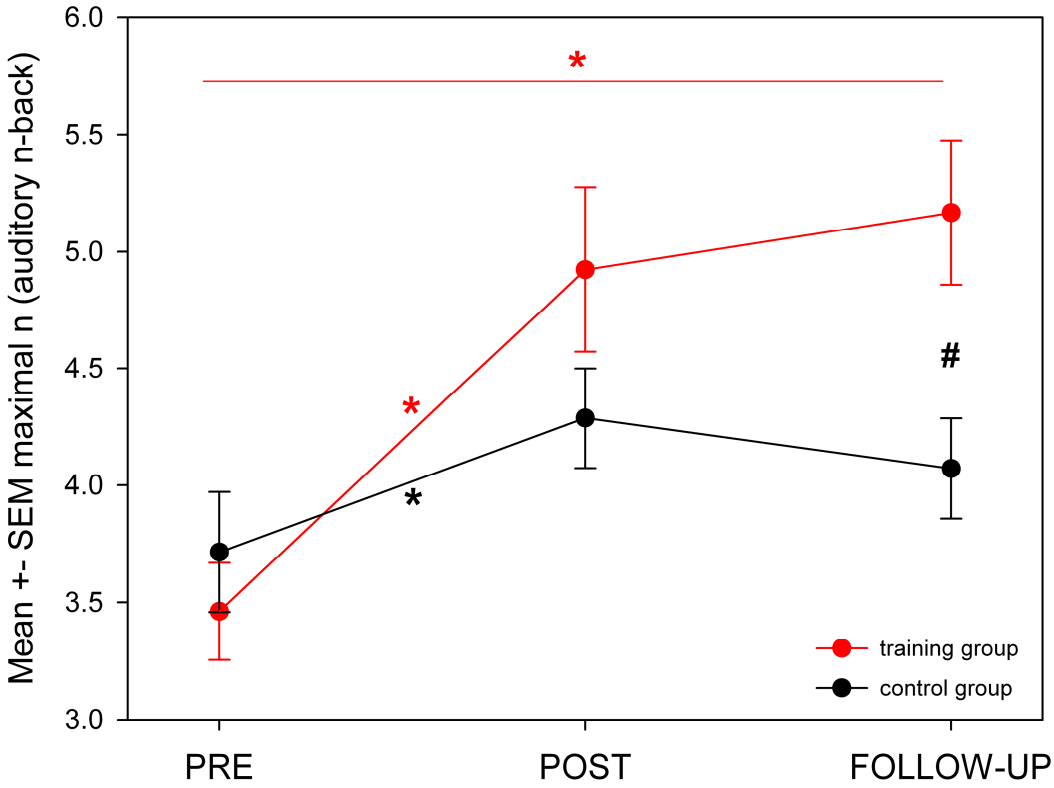

Figure 4.2.3: Mean ± SEM of maximal n (auditory n-back, ANB) per group and test session (PRE, POST, FU). The training group showed a significant increase from PRE to POST and to FU. The control group showed a significant increase from PRE to POST, but not FU. * indicate significant changes within group (training (red), control (black); paired t-test, $p < 0.05$. # indicate significant performance difference at the respective test session (unpaired t-test, $p < 0.05$).

In a following step, we analyzed the improvements in ANB in relation to the training gain and amount. Training gain, measured by the difference in performance between the first session and the session of maximal performance (Pearson correlation, r = 0.76, $p < 0.05$, Fig. 4.2.4) as well as the difference between the first and the last session (Pearson correlation, r = 0.77, $p < 0.05$), correlated positively with the improvements from PRE to POST in ANB. No correlation between ANB increase and training amount was found.

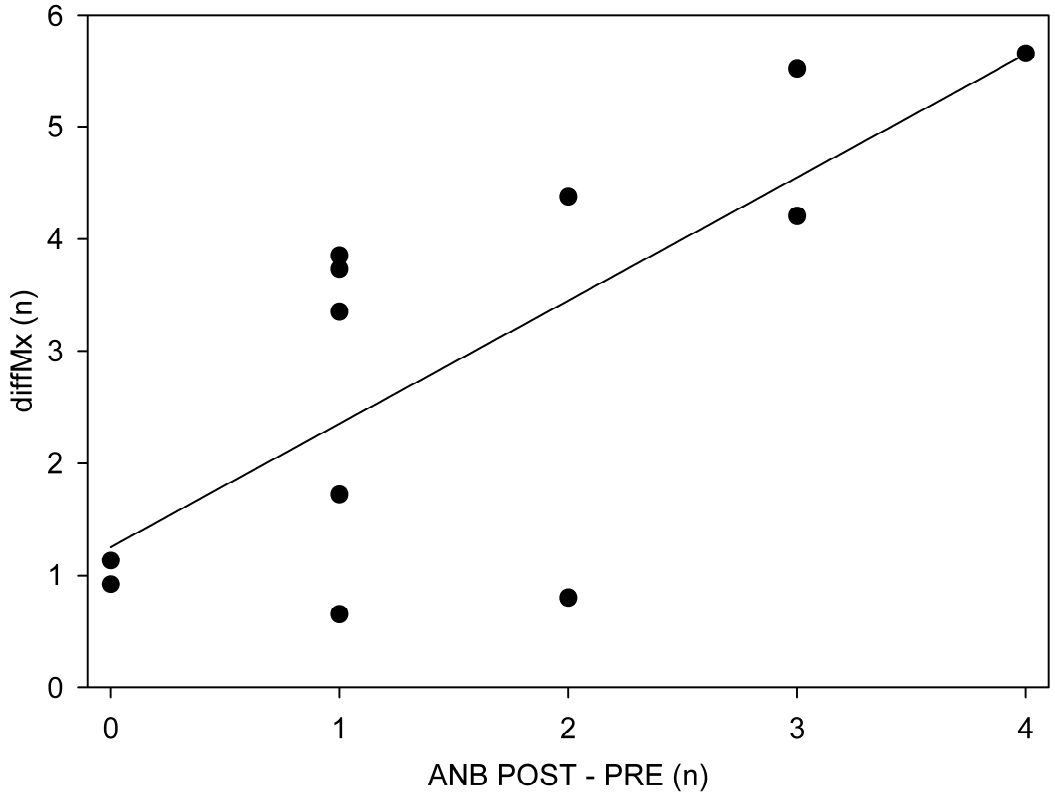

Figure 4.2.4: Association between visuospatial training performance increase and auditory n-back (ANB) performance increase. Correlation of training gain (diffMx, difference between Maximal Performance and performance at the first training session) with the change in ANB from PRE to POST (r = 0.76, $p < 0.05$).

Individual training performance

For assessing how training performance gain may influence the gain in auditory n-back (ANB), we grouped the subjects according to their training performance; by correlating the training performance over time with the session number (Fig. 4.2.5).

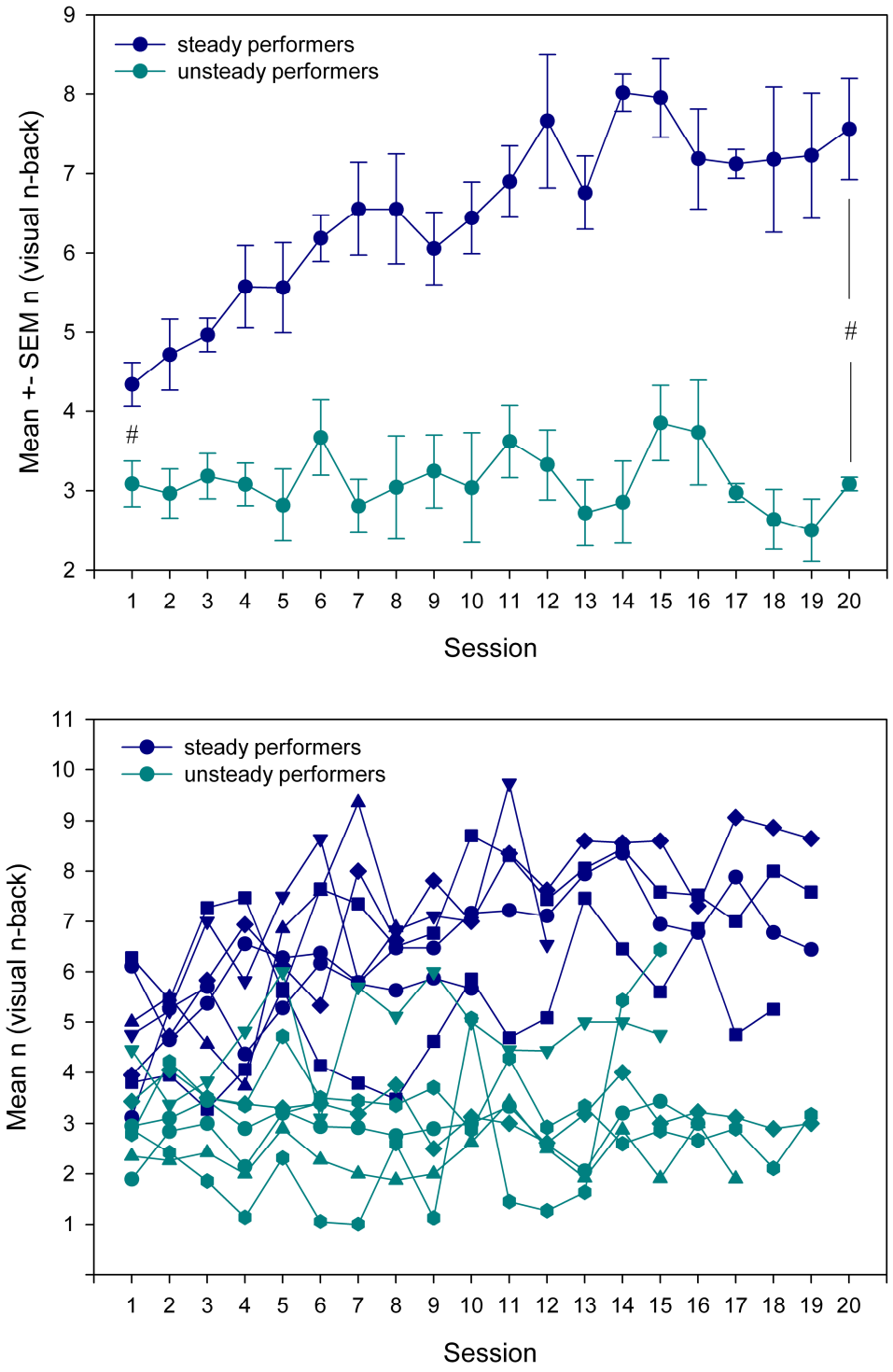

Figure 4.2.5: Visuospatial training performance for the steady and unsteady performers. Steady performers: individuals with a significant positive correlation between mean training performance (mean n) per session and the training session. Unsteady performers: no correlation. Top: mean ± SEM of n [visuospatial training task (VNB)] over each training session per group. Bottom: individual mean n (VNB) per training session. Blue: steady performer group. Green: unsteady performer group. # indicates significant performance differences between steady and unsteady performers at the first and the last session (unpaired t-test, $p < 0.05$).

In seven of the 14 training subjects, training days positively correlated with daily performance, indicating a steady increase of training performance over the entire training period (steady performer group). In the remaining subjects, the training performance was not stable and/or decreased (unsteady performer group).

In addition to the steady increase, the steady performers started on a significantly higher level (Fig. 4.2.5) and showed a larger increase in training performance from the first session to the session of maximal performance and to the performance at the last session (unpaired t-test, $p < 0.05$).

When we then compared ANB performance between these two groups, we found that the steady performers showed a significant higher increase from PRE to POST and higher increase in maximal ANB performance at POST and FU compared to the unsteady performers (Fig. 4.2.6).

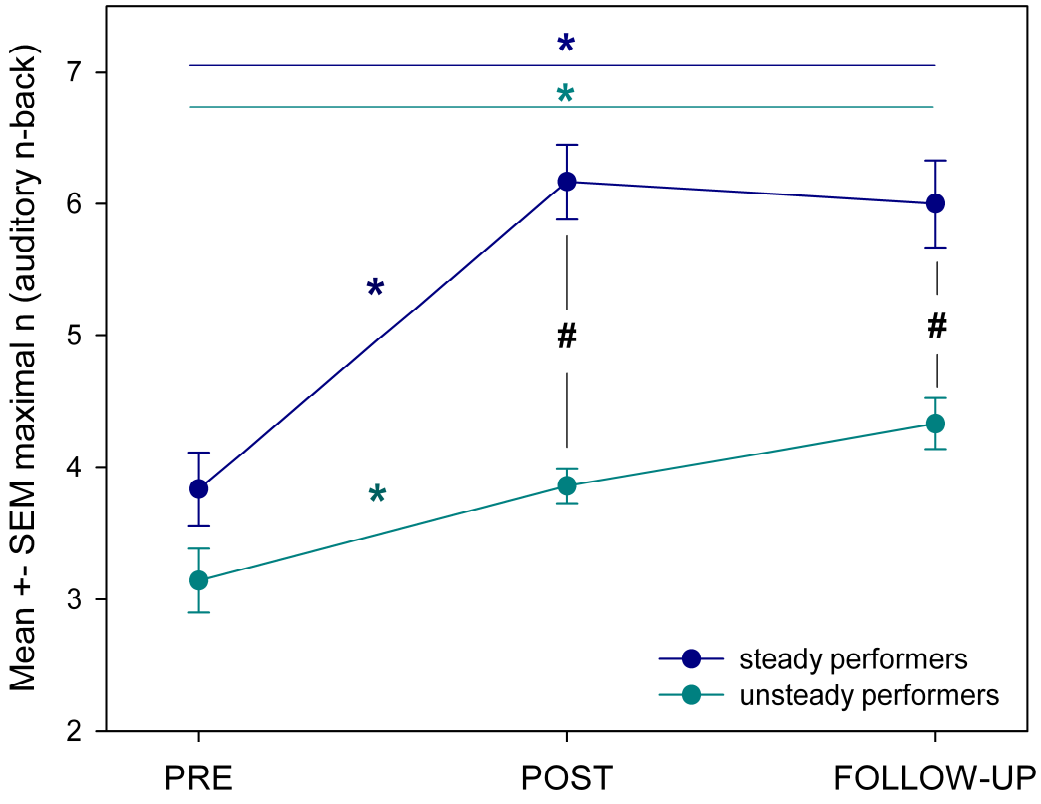

Figure 4.2.6: Mean ± SEM in auditory n-back (ANB) for the steady and unsteady training performers. * indicates significant changes within groups [steady performers (blue), unsteady performers (green)]. # indicates p < 0.05 (unpaired t-test between sessions (PRE, POST, FU)).

4.2.4. Discussion

During three weeks of visuospatial memory training, the participants in this study showed a significant performance increase in auditory working memory compared to passive controls. The better performance remained high after some months. The performance improvement correlated with the training quality, that is the performance

level, rather than with the amount of training. The cognitive control, measured with the Stroop and the Flanker task, was not associated with the performance increase. No significant transfer effects on fluid intelligence were observed.

Visual n-back training improves related working memory task performance

The strongest effect of VNB training was found on ANB, a working memory task closely related to the one trained for by our participants. Importantly, not the amount of training but the training gain correlated positively with the immediate and long-term increase in the auditory working memory task: the higher the increase in ANB from PRE to POST and to FU, the higher the training gain. Our approach based on correlating the individual training sessions with the training performance at each session supports the finding that training performance rather than the amount of training is crucial for the increase in ANB. The correlations show that the participants with a steady increase of their training performance had larger gains in training performance as well as in immediate and long-term ANB performances.

In agreement with our results, few other studies reported long-term effects of working memory training on the working memory performance (e.g., Dahlin et al., 2008; Li et al., 2008). In young and old adults, n-back task performance was maintained three months after spatial working memory training (Li et al., 2008). Dahlin et al. found stable improvements even eighteen months after the training (Dahlin et al., 2008). One explanation for such long-term effects in auditory working memory after training might be that training leads to more efficient use of working memory in daily life not only during the sessions, but also afterwards. A relationship between the efficient use of working memory and long term benefits for cognitive performance is supported by the observation that working memory tasks such as the digit span task and the visual-spatial working memory task were shown to predict mathematical and reading skills achieved in the first school years (Bull, Espy, & Wiebe, 2008). The transfer from test setting to daily life would be a convincing rationale to apply working memory training as a therapeutic tool. Indeed, in children with poor working memory performance, Holmes et al. found significant performance increases in mathematics 6 months after an intensive working memory training (Holmes, Gathercole, & Dunning, 2009). Thus, long-term measures of cognitive performance may indicate a certain effectiveness of cognitive training. If intensive working memory training is effective, a more efficient use of working memory would transfer into daily life, for example resulting in higher grades at school.

The idea that the observed improvements in visuospatial and auditory working memory would have occurred due to a use-dependent general increase in working memory capacity, may still be questioned by our finding that the letter-number sequencing, a typical task assessing working memory capacity, was not affected by training. This finding is in line with other studies comparing working memory capac-

ity (e.g., by digit span tasks) and n-back tasks (Jaeggi et al., 2008; Kane et al., 2007). As Kane et al. suggested (Kane et al., 2007), n-back tasks may 'not reflect primarily a single construct' because 'complex span tasks typically demand serial recall', whereas 'n-back tasks typically demand recognition'. Thus, serial recall and recognition do not correlate. This observation may indicate that the auditory and visuospatial tasks are actually too similar to draw conclusions about general effects on working memory *per se*.

Working memory training does not significantly improve fluid intelligence

We found no significant effects of the training on non-verbal matrices test when comparing the training group to the control group. Also Jaeggi et al., when comparing fluid intelligence test performance between the experimental and control group and between three test sessions, did not find any effect of visuospatial working memory training on Gf (Jaeggi, Buschkuehl et al., 2011). Only after grouping the participants according to their training gain in a large and small training gain group (median split) some differences in transfer effects were observed: immediate transfer effects and, to lesser extent, long-term transfer effects were significantly higher in the large training gain group compared to the low training gain and control group. When we performed the same median split in our training group, we were not able to find any statistically significant transfer effects. However, several aspects, such as the training duration and the attractiveness of the training, render it difficult to directly compare the two studies. After all, in adults, the transfer effects of working memory training on fluid intelligence could not be replicated (Redick et al., 2013). Furthermore, in a recently presented meta-analysis that investigated the effects of working memory training on cognitive tasks, Melby-Lervåg and Hulme revealed that working memory training indeed improves the working memory capacity immediately after the training. The training however does not lead to transfer effects on fluid intelligence when focusing on studies with control groups and randomization (Melby-Lervåg & Hulme, 2013). Thus, despite the low number of participants included in this study, our negative finding concerning transfer effects on fluid intelligence is congruent with the current literature.

Working memory training does not affect processing speed and is not related to cognitive control

As proposed by Diamond and Lee (Diamond & Lee, 2011), working memory training does not improve inhibition or processing speed. Our data are in line with this notion, since we did not find any effects of the training on the processing speed tasks and the Stroop and Flanker test. The training and the control groups increased processing speed over the three sessions similarly. As shown by Conway et al., digit-symbol tasks may rather reflect short-term memory than working memory (Conway,

Cowan, Bunting, Therriault, & Minkoff, 2002). However, our short-term memory task was not affected by working memory training. Thus, we conclude that processing speed, used for measuring digit-symbol and symbol search tasks, are unaffected by working memory training and/or practice effects may be stronger.

Our data did not indicate that cognitive control, measured with the Stroop and Flanker task, is associated with the training gain and effects on auditory working memory. Although our results do not corroborate the theory of attentional control being a crucial factor for working memory (Kane & Engle, 2003), we do not question the necessity of cognitive control for reasoning. Again, with our data sample of limited size and large inter-individual variability we may not have been able to capture factors that influence working memory performance gains or even changes in cognitive functions due to training.

We conclude from these results that the performance increase in a visuospatial working memory task is beneficial for auditory working memory in children and adolescents. The dominant long-term effects underline the importance of assessing performance not only right after the cognitive training, but also several months later. Regarding the importance of working memory for other cognitive functions, studies comparing populations of different developmental stage or of low and high working memory capacity are needed.

4.3. Local increase of sleep slow wave activity after three weeks of working memory training in children and adolescents

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Author contributions MW, PA, OGJ and RH conceived the study. Together with FP and AM, they designed the study. Recruitment of participants and data assessment were done by FP and AM. Analysis was performed by FP. FP and RH wrote the first draft of the manuscript. All authors were involved in the revision of the draft manuscript and have agreed to the final content.

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Under review

Abstract Evidence is accumulating that EEG sleep slow wave activity (SWA), the key characteristic of deep sleep, is regulated not only globally, but also locally. Several studies have shown local learning- and use-dependent changes in SWA. In vitro and in vivo animal experiments and studies in humans indicate that these local changes in SWA reflect synaptic plasticity. During maturation, when synaptic plastic changes are most prominent, learning is of utmost importance. Thus, in this study, we aimed to assess the effects of three weeks of working memory training on sleep SWA topography using high-density EEG recordings in children and early adolescents (N = 28). Our results show that after intensive working memory training, sleep SWA was increased in a small left frontoparietal cluster, (11.06 ± 1.24 %, mean ± SEM). In addition, the local increase correlated positively with working memory performance assessed immediately (r = 0.66) and two to five months (r = 0.68) after the training. Our results indicate that the increase in SWA reflects cognitive training-induced plasticity in a region known to be involved in working memory performance. Thus, in the future, the mapping of sleep SWA may be used to longitudinally monitor the effects of working memory training in children and adolescents having working memory deficiencies.

4.3.1. Introduction

Sleep slow wave activity (SWA, EEG power between 0.5 to 4.5 Hz), a major characteristic of NREM sleep, is globally regulated by a homeostatic process: in parallel to sleep need, SWA increases during wakefulness and decreases during sleep (Achermann & Borbély, 2011). Animal (e.g., Bushey et al., 2011; Vyazovskiy et al., 2008) and computational (e.g., Olcese et al., 2010) studies indicate that these changes in SWA are driven by synaptic plasticity, i.e. changes in synaptic strength (Esser et al., 2007; Riedner et al., 2007; Vyazovskiy et al., 2007). For example, wakefulness in rats was associated with a net increase of electrophysiological and molecular markers of synaptic strength. These markers were renormalized during sleep (Vyazovskiy et al., 2008). Moreover, a recent study found also sleep-wake dependent structural changes, which seem to be most pronounced during development: In adolescent mice, wakefulness was accompanied by a net spine gain and sleep by a net spine loss (Maret et al., 2011).

Also in humans, evidence is accumulating that sleep SWA and synaptic plasticity are associated since it has been shown that sleep is regulated locally in a use- and experience-dependent manner (e.g., Huber et al., 2004; Kattler, Dijk, & Borbely, 1994). For instance, a local increase of SWA was observed in response to sleep deprivation in predominantly frontal regions which are thought to be the most used areas in the adult brain (Cajochen, Foy et al., 1999; Finelli et al., 2001; Horne, 1993). More recently, local changes in SWA were associated with learning processes involving specific brain regions (e.g., Huber et al., 2004). As shown by Huber et al., learning a visuomotor adaptation task in the evening induced a local increase in SWA over the right parietal cortex during subsequent sleep (Huber et al., 2004). In fact, a PET study showed that this area is crucial for visuomotor learning during wakefulness (Ghilardi et al., 2000). Also other studies indicated that learning induced plastic changes result in corresponding changes of SWA during subsequent sleep (Landsness et al., 2009; Maatta et al., 2010).

SWA also reflects plastic changes during development (e.g., Kurth et al., 2010), when large modifications take place. Such developmental changes of gray matter encompass synapse formation during early childhood and elimination during late childhood and adolescence (Huttenlocher, 1979; Huttenlocher & Dabholkar, 1997). Magnetic resonance imaging studies showed a spatiotemporal pattern of how gray matter density changes across development: frontal areas show later maturation than more occipital areas (Gogtay et al., 2004). Accordingly, sleep EEG data from childhood to late adolescence reflect brain maturational changes, indicated by an overall decrease of SWA (Campbell & Feinberg, 2009) and a topographical shift of maximal SWA from more occipital to frontal regions (Kurth et al., 2010).

Studies investigating local changes in SWA focused on either immediate effects of before-sleep-manipulations in adults (e.g., Huber et al., 2004) or on very long-term, age-dependent changes during development (e.g., Kurth et al., 2010). However, no study so far investigated longitudinally learning-induced use-dependent changes of SWA in relation to performance, i.e. after several weeks of training or months thereafter. Thus, in our study we were interested to see how a working memory training during three weeks changes SWA locally. Working memory is a fundamental cognitive function that describes the ability to maintain information while processing unrelated mental operations (Baddeley, 2003). We applied working memory training, because it was shown to be beneficial for working memory performance not only in adults (Buschkuehl et al., 2008; Jaeggi et al., 2008), but also in children and adolescents (Pugin et al., under review). We demonstrated in a recent study, that visuospatial n-back training serves to assess learning processes in young subjects (10 to 16 years), since we found significant working memory performance changes immediately and even months after the training (Pugin et al., under review). In this study, we used high-density (HD) EEG recordings to investigate local changes in SWA induced by the visuospatial n-back training in the same young population as presented in Pugin et al.. At this age range, cortical and cognitive changes are most prominent (e.g., Huttenlocher, 1979; Luna et al., 2004; Shaw et al., 2006). As shown in previous studies (e.g., Huber et al., 2004; Kurth et al., 2010; Ringli et al., 2012), HD EEG recordings allow to detect local changes in SWA with high spatial resolution (128 electrodes; for review, see Lustenberger & Huber, 2012). Moreover, we tested whether local changes in SWA would be predictive for performance changes in auditory n-back performance, a working memory task found to be improved after working memory training.

4.3.2. Methods

Participants

Fourteen male subjects participated in the study (12.97 ± 0.40 years old, mean ± SEM; range 10 years and 4 months to 16 years and 2 months). The inclusion criteria were male, right-handed (1 ambidexter), good sleep and regular sleep-wake times, no cognitive or learning disabilities, non-smokers, moderate caffeine and alcohol consumption (monitored by sleep log, see below), no diseases or intake of medication, no flights crossing more than two time zones in the last six months and no regular daytime sleep. Parents and subjects gave written consent after careful introduction to the study. The study was approved by the local ethics committee (Canton of Zurich, Switzerland).

At least three days prior to both overnight sleep sessions at the sleep laboratory, subjects were asked to maintain their habitual sleep-wake schedule which was checked by wrist motor actigraphs and self-reported sleep-wake logs. In the sleep log, besides

sleep and wake times, subjects were asked to write down daily amount of caffeine and alcohol intake as well as day time naps and physical activities.

Experimental design

Sleep was recorded twice at the sleep facilities of the University Children's Hospital Zurich (PRE and POST training, Fig. 4.3.1) with three weeks of training in between.

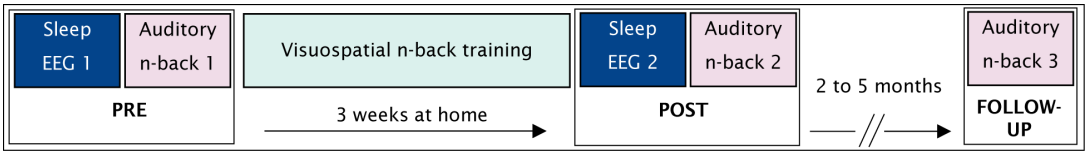

Figure 4.3.1. Study design and timeline. In a first session before training (PRE), high-density sleep EEG was recorded overnight and in the morning, subjects performed the auditory n-back task (auditory n-back 1). After three weeks of visuospatial n-back training, the procedure at PRE was repeated (POST training). In a follow-up session after 2 to 5 months, auditory n-back performance was reassessed (auditory n-back 3).

In the morning after the first night (PRE), subjects performed an auditory n-back (ANB) task for 10 minutes (Buschkuehl et al., 2007). After this ANB session, the subjects started an intensive working memory training for three weeks until the next overnight sleep session (POST training). Training performance was supervised at the first session and at one home visit within the three weeks. Immediately (POST) and several months after the training (follow-up, FU), auditory n-back performance was reassessed (for more methodological details, see Pugin et al., under review).

Intervention: Computerized visuospatial n-back training (*BrainTwister* software)

The training consisted of a visuospatial n-back task, developed by the University of Berne (Buschkuehl et al., 2007). In a continuous series of stimuli, i.e. blue squares appearing on a computer screen, subjects had to indicate by pressing a button when the position of the blue square was in the same position as n stimuli before. In other words, for each stimulus in a trial (series of 20 + n stimuli), the position of the current stimulus had to be compared to the stimulus presented n stimuli before. Thus, the larger the n, the more correct positions were memorized. The working memory task was adaptive, thus, the difficulty level, i.e. the n, was adjusted to the individual performance after every trial. For each trial, subject's performance was saved and was retraced during the home visit as well as retrospectively after the training period. Training duration lasted 30 minutes per day (maximally 20 days).

Working memory task: Computerized auditory n-back task (*BrainTwister* software)

The adaptive auditory n-back (ANB, University of Berne) task was similar to the visuospatial training task, but with a computerized voice speaking letters instead of visual squares appearing on the screen. ANB was assessed at PRE, POST and FU

and lasted 10 minutes at each session. The maximally reached n, thus, the maximal number of letters that could be memorized, was used for analysis for further details on the working memory task, see (for further details on the working memory task, see Pugin et al., under review).

Sleep EEG recording and analysis

All fourteen subjects, all-night sleep EEG, electrooculogram and electromyogram were recorded at the sleep facilities of the University Children's Hospital Zurich (Zurich, Switzerland). Sleep was recorded with high-density (HD) EEG (Electrical Geodesics Sensor Net for long-term monitoring, 128 channels, referenced to a vertex electrode). Prior sleep, electrodes were prepared that impedances were below 50 kΩ. Preprocessing of sleep data was performed in Matlab 7.7.0 (R2008b). Data were sampled at 500 Hz (filtered 0.01 - 200 Hz). Offline, after high-pass (0.05 Hz) and low-pass (50 Hz) filtering, data were down sampled to 128 Hz. Power spectral density for each derivation and night was estimated with Welch's method (Matlab function pwelch, average of five 4 s-epochs, Hanning window). With semi-automatic artifact detection, derivations and epochs were rejected automatically on a 20 s basis when exceeding a threshold based on a mean power value in the 0.75 to 4.5 and 20 to 30 Hz bands or by visual inspection. Each derivation was average referenced to the mean of all unrejected channels. The EEG signal was visually scored according to the standard criteria of the AASM (American Academy of Sleep Medicine; Iber et al., 2007). For each subject, lights-off time was determined according to their habitual sleep time. Wake-up time had to be adjusted to the subjects' needs (e.g., school start). Due to inter-individual differences in sleep duration, we analyzed the minimum common number of NREM-REM sleep cycles found in all subjects and nights (3 cycles). Definition of sleep cycles was performed according to Feinberg and Floyd (Feinberg & Floyd, 1979). As described by Kurth et al. (Kurth et al., 2010), skipped REM episodes were adjusted if necessary.

SWA (EEG power between 1 and 4.5 Hz) was calculated for each subject for both nights (PRE, POST). Power values were normalized for each subject and night by dividing SWA in each derivation by the mean SWA over all derivations. For power density spectrum, power density values for each frequency bin (0.25 Hz) were normalized for each subject and night by dividing power density in each derivation by the mean power over all derivations.

In order to localize the electrodes' position over respective brain regions, electrodes were digitized and co-registered to the subject's MRI using SofTaxic Optic (EMS Inc.) and the three dimensional optical digitizer (Polaris Vicra; Northern Digital). With the Talairach Client, cortical brain regions underlying the single electrodes were estimated as applied previously (e.g., Kurth et al., 2010; Ringli et al., 2012).

Statistics

Statistics was performed with SPSS (PASW Statistics 18) and Matlab 7.7.0 (R2008b). In order to detect local changes in SWA related to the training, we performed paired t-test comparing PRE and POST training. To control for multiple comparisons, statistical nonparametric mapping (SnPM) was applied (Huber et al., 2004; Nichols & Holmes, 2002; Ringli et al., 2012). Power spectral density in each bin (0.25 Hz) between the two nights (PRE, POST training) was compared by paired t-test. Similar to Stadelmann et al., only ≥ 5 consecutive frequency bins were considered for interpretation (Stadelmann et al., 2013). A backward regression model performed in SPSS 18 was employed to determine the most appropriate model that predicts the dependent variable (Agresti & Finlay, 2008). More specifically, this way we determined how much of the variance in the dependent variable (ANB performance after the training) may be explained by the predictor variables (age, baseline ANB performance at PRE training and changes in SWA). The model with the highest adjusted R^2 was chosen. Beta (ß)-values (standard deviation units) describe the relationship between predictors and the dependent variables (i.e., the amount of change in the dependent variable if the predictor changes approximately one standard deviation).

4.3.3. Results

Performance changes working memory

As described in Pugin et al. (Pugin et al., under review), intense training resulted in significant immediate and long-term improvements in auditory n-back performance.

Local increase in SWA after three weeks of visuospatial n-back training

In a first step, we compared sleep quality and sleep stages between the two nights. Neither sleep efficiency nor the amount of NREM sleep stages 1 to 3 differed between PRE and POST training nights (Table 4.3.1). Only REM sleep (duration and percentage) was increased in the POST training night compared to the PRE training night.

	PRE N = 14		**POST** N = 14			
Sleep variables	Mean	± SEM	Mean	± SEM	t	*p*
Sleep latency (min)	11.4	1.7	11.9	2.5	-0.2	0.86
Wake after sleep onset (min)	7.9	1.6	5.3	1.5	1.1	0.27
Sleep efficiency (%)	89.6	2.1	94.1	1.0	-1.7	0.11
REM sleep latency (min)	155.7	10.8	136.6	13.6	1.5	0.17
NREM sleep stage 1 (min)	11.5	1.7	10.0	1.3	0.9	0.37
NREM sleep stage 1 (%)	4.6	0.6	3.9	0.5	1.3	0.21
NREM sleep stage 2 (min)	124.5	8.4	128.8	8.8	-0.3	0.73
NREM sleep stage 2 (%)	50.1	1.9	50.5	2.9	-0.1	0.89
NREM sleep stage 3 (min)	95.5	5.7	95.8	8.7	0.0	0.97
NREM sleep stage 3 (%)	39.1	2.1	37.5	2.9	0.6	0.56
REM sleep (min)	15.8	2.3	21.1	2.6	-2.3	0.04 *
REM sleep (%)	6.2	0.7	8.1	0.8	-2.8	0.02 *
Total sleep time (min)	247.3	11.2	255.7	8.5	-0.6	0.54
Total time in bed (min)	279.4	16.9	272.0	9.2	0.4	0.71

Table 4.3.1: Sleep variables derived from visual scoring for PRE and POST training nights (see Fig. 4.3.1). Sleep latency: time from lights-off to first appearance of NREM sleep stage 2, 3 or REM sleep. Sleep efficiency: Total sleep time as percentage of time in bed. Amount of scored NREM and REM sleep stage relative to the total of the first three NREM-REM sleep cycles (in min and percentage). Total sleep time: Minutes scored asleep (NREM sleep stage 1, 2, 3 and REM sleep). Total time in bed: time spent in bed from lights-off to lights-on. * indicates significant difference between PRE and POST training nights (paired t-test, $p < 0.05$).

Next, we analyzed regional training-related changes in SWA by comparing sleep SWA topography before and after the training (PRE, POST). SWA topography (Fig. 4.3.2 left) was similar for the two nights (PRE, POST), with both nights showing an age-typical SWA maximum over frontocentral regions (Kurth et al., 2010). Statistical non-parametric mapping (SnPM) permutation test revealed a significant increase in SWA in three derivations over the left frontal and parietal cortex, including inferior frontal gyrus, precentral and postcentral gyrus, as well as in one derivation over right prefrontal gyrus (Fig. 4.3.2 right).

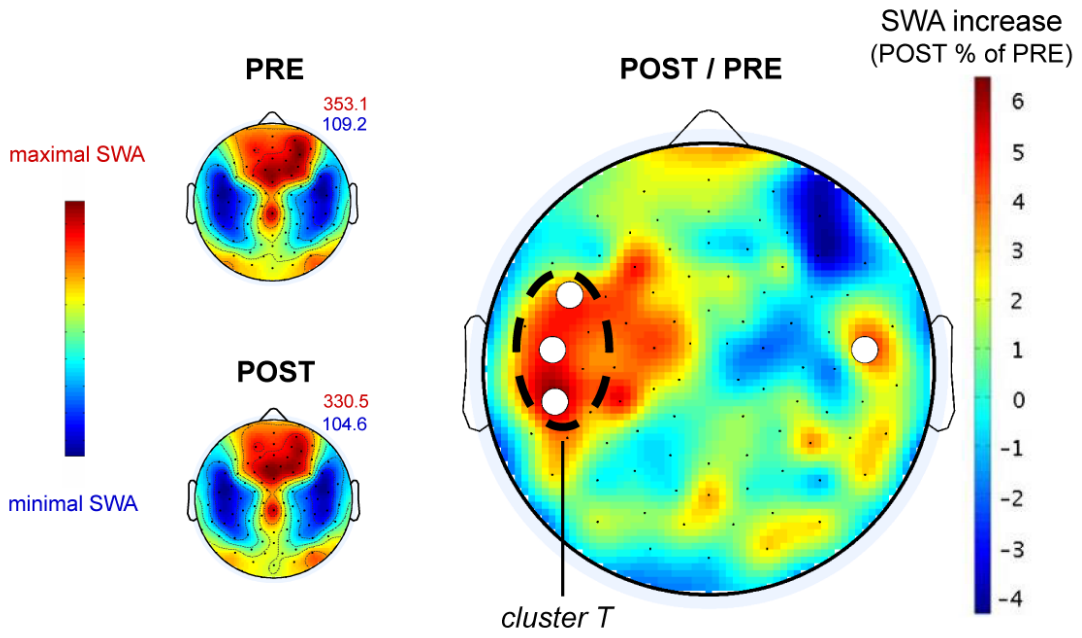

Figure 4.3.2. Left: Spatial distribution of slow wave activity (SWA) for the night before (PRE) and after (POST) working memory training. Blue to red color indicate minimal to maximal SWA. The numbers in red and blue indicate minimal and maximal absolute mean power density ([μV^2 Hz^{-1}]) values for PRE and POST training recordings. Right: Mean SWA change from PRE to POST in percentage of the PRE recording. The white dots indicate derivations where SWA was significantly increased from PRE to POST (statistical non-parametric mapping, see Methods for details).

We considered the increase in the left three derivations as a cluster (*cluster T*). Similar to Huber et al. (Huber et al., 2004), for each individual the derivation with a maximal power increase from PRE to POST within this cluster was determined and used for further analysis. The average increase in *cluster T* from PRE to POST (ratio POST / PRE) was 11.06 ± 1.24 % (mean ± SEM).

Local increase in SWA is related to auditory n-back performance after the training

In order to assess whether the improved performance in an auditory n-back (ANB) task performed at POST and FU was related to the local increase in SWA, we correlated ANB performance with the maximum SWA increase in *cluster T*. A positive association between the SWA increase and ANB POST and ANB FU was observed (Fig. 4.3.3). No significant correlation was found at the first session (PRE).

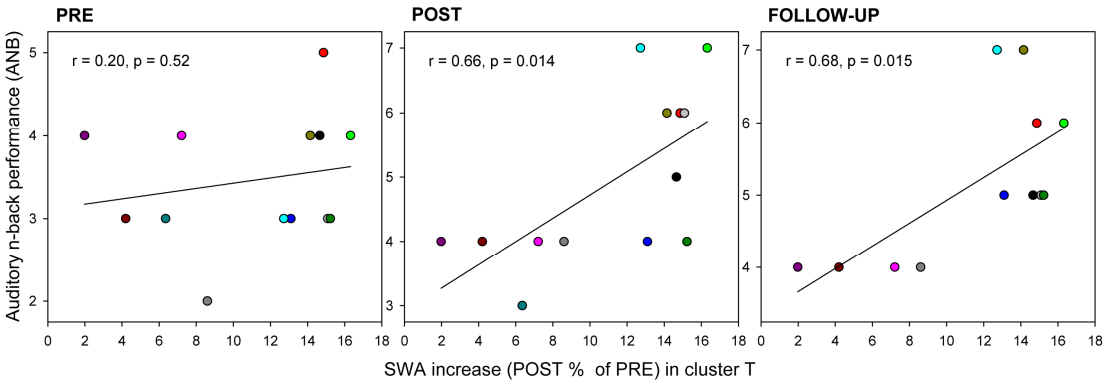

Figure 4.3.3. Pearson correlation between SWA increase (%) from PRE to POST in *cluster T* (three left derivations; see Fig. 4.3.2) with maximal auditory n-back (ANB) performance at PRE (left) and POST (middle) training and at follow-up (FU; right). Colors indicate individual data points per session (PRE, POST, FU). The n indicates the maximal performance reached at this session, i.e. 2 to 7-back.

With a backward regression analysis (see Methods for details), we aimed to reveal how predictive the local increase in SWA was for performance in ANB right after the training (POST) and some months later (FU). Besides the SWA increase in *cluster T*, the predictors were age and ANB performance at PRE. For ANB POST performance, the model including both ANB PRE performance and the local increase of SWA in *cluster T* showed the highest adjusted R^2 (adjusted $R^2 = 0.39$, $F(1, 11) = 4.84$, $p < 0.05$; ß (ANB PRE) 24.3 %; ß (SWA increase in *cluster T*) 61.2 %, Table 4.3.2). Thus, ANB performance at POST is not predicted by age but by ANB PRE and, to a larger extent, by the SWA increase in *cluster T*. ANB FU performance was not predicted by age and baseline performance (ANB PRE) but by the local increase in SWA in *cluster T* (adjusted $R^2 = 0.41$, $F(1,10) = 8.53$, $p < 0.05$; ß = 67.9 %, Table 4.3.2).

Dependent variable		*Constant* and Predictor(s)	B	SE B	ß
ANB POST	Step 1	*Constant*	-17.22		
		Age	-.00	.00	-.29
		ANB PRE	.59	.43	.34
		SWA increase *Cluster T*	20.93	7.67	.76 *
	Step 2	*Constant*	-15.18		
		ANB PRE	.41	.39	.24
		SWA increase *Cluster T*	16.80	6.31	.61 *
	Step 3	*Constant*	-15.19	6.92	
		SWA increase *Cluster T*	18.11	6.22	.67 *
ANB FU	Step 1	*Constant*	-12.56		
		Age	-.00	.00	.01
		ANB PRE	.29	.39	.21
		SWA increase *Cluster T*	14.91	7.05	.64 #
	Step 2	*Constant*	-12.62		
		ANB PRE	.29	.33	.21
		SWA increase *Cluster T*	15.04	5.53	.65 *
	Step 3	*Constant*	-12.42	6.03	
		SWA increase *Cluster T*	15.77	5.40	.68 *

Table 4.3.2. Backward regression analysis. Auditory n-back (ANB) performance at POST is predicted by ANB PRE and the local increase in SWA (*cluster T* (see Fig. 4.3.2), step 2: adjuster $R^2 = 0.39$). The standardized Beta (SE B) at step 2 is higher for the SWA in *cluster T* than ANB PRE, suggesting that the former is more important for the model. ANB performance at FU is predicted by the local increase in SWA (*cluster T*, step 3: adjusted $R^2 = 0.41$). ANB POST: $R^2 = .54$, .49 and .44 for Step 1, 2 and 3. ANB FU: $R^2 = .50$, .50 and 0.46 for Step 1, 2 and 3. ANOVA *p*-values for the models: * and # indicate $p < 0.05$ resp. $p < 0.1$.

Increase in spectral power within cluster T in theta and sigma range

To check for frequency specificity of the local changes in SWA, we investigated the normalized power spectrum for derivations in *cluster T* (Fig. 4.3.4). This analysis revealed a significant increase in the slow wave frequency band (0.5 to 4.75 Hz) and in the theta range (7.25 to 8.75 Hz) from PRE to POST training.

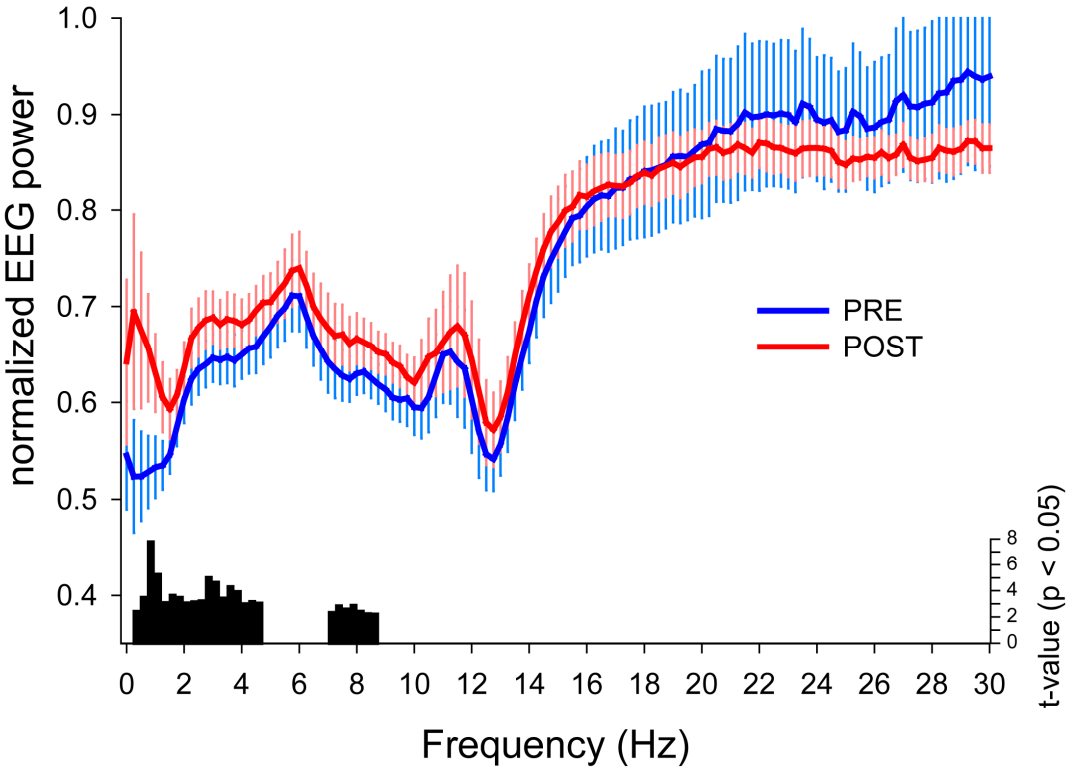

Figure 4.3.4: Power density spectrum (1 to 30 Hz, bin width = 0.25 Hz) for the derivation showing maximal power in *cluster T* (see Fig. 4.3.2) during the PRE (blue) and POST (red) training night (mean ± SEM). Power density values for the derivations in *cluster T* were expressed relative to the mean power density across all derivations (see Methods for details about this normalization). Below: Black vertical bars indicate significant t-values of paired t-tests per bin comparing normalized power density values of PRE and POST training nights.

4.3.4. Discussion

We observed a significant increase in SWA in three left frontoparietal derivations (*cluster T*) after cognitive training. After three weeks of visuospatial working memory training, this local increase correlated with performance in an auditory n-back (ANB) task immediately (POST) and some months after the training (FU). Regression analysis revealed that auditory n-back performance variance after the training was best explained by the local increase in SWA.

We would like to highlight three major aspects of our study. First, we investigated not the immediate effects of learning on subsequent sleep EEG, but the effects after several weeks of training. Previous studies investigated learning-induced SWA changes, in children (Wilhelm et al., 2013) and as well as in adults (e.g., Huber et al., 2004). In these studies, the learning task was performed the day before the sleep EEG recording, with the aim to detect performance-related effects in the subsequent sleep EEG recording. In fact, local changes in SWA as a result of intense use or learning were observed (Huber et al., 2004; Landsness et al., 2009; Maatta et al.,

2010). Our data show that local changes in SWA are also observable after a longer period of learning (3 weeks). Functionally, the increase in SWA may have resulted from a steady increase in synaptic efficacy over the three weeks of training, reflected by the induction of learning-related long-term potentiation (LTP) and the synthesis of proteins involved in structural remodeling of synapses (for review, see Benington & Frank, 2003). Different forms of plasticity were related to sleep. In rats, Vyazovskiy et al. (Vyazovskiy et al., 2008) showed that synaptic potentiation dominates during wake, which was indicated by a net increase in GluR1-containing AMPA receptor levels and an increased phosphorylation of receptors and kinases involved in learning and plasticity. Synaptic strength may be described as the efficacy of excitatory cortical connections, characterized by firing rate and synchrony (Vyazovskiy et al., 2009). In other words, the higher the synaptic strength, e.g., after learning during wake and long-term potentiation (LTP), the higher the SWA in the used region in the following sleep episode. Also structural changes such as the number of spines were related to sleep (Maret et al., 2011). These studies support the hypothesis that the intensive working memory training over three weeks may have led to LTP-like or structural plastic changes in a distinct network. As a consequence firing rates and synchrony during deep sleep were altered, resulting in higher SWA, particularly in the three left frontoparietal derivations over cortical areas involved in working memory training. Those functional and structural changes would in turn be reflected in performance changes, building the basis for the observed sustained working memory performance increase over several months. Thus, the local increase of SWA over the left frontoparietal cortex may reflect the 'ability to be plastic' also *after* the intervention, thereby predicting later outcome of cognitive performance.

Second, this is the first study showing changes in sleep SWA after working memory training. So far, studies have reported about local changes in SWA after visuomotor or sensorimotor manipulations (Huber et al., 2006; Huber et al., 2004; Landsness et al., 2009; Maatta et al., 2010), but not after manipulations of higher cognitive functions. The investigation of local SWA changes in relation to complex cognitive functions such as working memory is challenging because of the high degree of underlying interconnected areas involved in cognitive functions. We found a local increase of SWA over a frontoparietal network that encompassed inferior frontal, precentral and postcentral gyri, including Brodmann areas (BA) 2, 6, 34, 44 and 45. This localization is in agreement with the literature showing cortical activation during n-back tasks in similar regions (Jaeggi et al., 2003; Owen, McMillan, Laird, & Bullmore, 2005). More specifically, activation in the left hemisphere during verbal working memory tasks is enhanced compared to activation during visual tasks (Owen et al., 2005). Thus, the left increase in SWA and its relation to auditory working memory performance may reflect plastic changes in an area involved in verbal processing. However, working memory processing relies on the activation of both hemispheres (Jaeggi et al., 2003; Owen et al., 2005). The increase in SWA in one right derivation

(see Fig. 4.3.2), contralaterally to *cluster T*, may point to such a bilateral activation. Thus, our findings support the notion that SWA can be used to map plasticity-driven changes. For example, in the ongoing debate about the effectiveness of working memory training on cognitive performance, mapping changes of SWA may serve as a promising tool to investigate long-term effects of working memory training, as an addition to the already established purely cognitive assessments or functional neuroimaging measures. Moreover, according to our model of the regression analysis, the increase in SWA in *cluster T* seems to be the strongest predictor variable for to ANB performance at POST and FU. Thus, our results show that local changes in SWA may even have some predictive value for training related changes in working memory performance, even months after the end of the training.

Finally, few studies investigated SWA changes due to learning in young populations. Recently, Wilhelm et al. reported learning-related changes in SWA in a broader, but overlapping age range (Wilhelm et al., 2013). They found an increase in SWA following a visuomotor learning task in a region similar to the one observed in Huber et al. in (Huber et al., 2004). Interestingly, this local increase was more pronounced in children than in adolescent and adult subjects and was correlated to the level of SWA in this particular region (Wilhelm et al., 2013). In line with this finding, the local increase of SWA after working memory training was larger for those subjects who showed higher SWA in this area prior to training (Pearson correlation between PRE SWA and the increase of SWA in the frontoparietal cluster: $r = 0.65$, $p = 0.013$). According to Kurth et al. (Kurth et al., 2010), frontoparietal regions show maximal SWA between 11 and 14 years which may indicate that these regions undergo major plastic changes during this period. As hypothesized by Wilhelm et al., these regions may be more prone to an intervention. This assumption fits to the concept of critical periods during development, as shown in young cats: at a specific time window for visual development, monocular eye deprivation showed NREM sleep-dependent cortical plasticity (Frank, Issa, & Stryker, 2001). In our study, the positive correlation of local SWA (*cluster T*) with auditory n-back performance after training (see Fig. 4.3.3) supports these ideas, because increased susceptibility for plastic changes, indicated by higher initial SWA and a larger increase in SWA, seems to be advantageous for later working memory performance (Fig. 4.3.3, Table 4.3.2: Regression analysis). Consequently, as working memory performance reaches adult-level performance around 20 years of age (Luna et al., 2004), older subjects may show less prominent SWA increase because a plasticity plateau is reached. Altogether, these observations indicate that developmental aspects may be relevant when investigating learning-related local changes in SWA.

However, some limitations have to be considered when interpreting the findings of our study. One issue is the small number of subjects within a rather broad age range. As a result, we cannot exclude that with more statistical power, for example, the single derivation showing increased SWA on the contralateral site of *cluster T* might

extend into a cluster. However, it is needless to say that such a demanding study protocol is challenging for a young subject population. We also would like to point out that the power spectral changes from night 1 to night 2 in *cluster T* were not restricted to the slow oscillation (< 1 Hz) and slow waves range (1 to 4.75 Hz), but were also found at 7.25 to 8.75 Hz. However, also other studies reported changes in theta range. For example, during recovery sleep after 40 hours of sleep deprivation both slow wave and theta activity was increased in frontal derivations (Cajochen, Foy et al., 1999). Also Huber et al. reported a parallel increase in theta activity after the visuomotor learning task (Huber et al., 2004).

In conclusion, we show for the first time that SWA can be used to map long-term effects of an intensive learning period in young subjects. Hereby, the local changes in SWA seem to be an indicator for learning-induced plasticity related to complex cognitive functions such as working memory. In the future, the mapping of SWA may be used to longitudinally monitor the effects of working memory training in children and adolescents with working memory deficiencies.

4.4. Brain tissue oxygen saturation increases during the night in adolescents

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Author contributions RH, PA and MW conceived the study. Together with AM and FP, they designed the study. Recruitment of participants and data assessment were done by AM and FP. Sleep data was processed by FP. Analysis was performed by AM. AM and MW wrote the first draft of the manuscript. All authors were involved in the revision of the draft manuscript and have agreed to the final content.

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Abstract How does the oxygen metabolism change during sleep? We aimed to measure the change in brain tissue oxygen saturation (StO_2) before and after sleep with near-infrared spectroscopy (NIRS) using an in-house developed sensor. According to the synaptic homeostasis hypothesis (Tononi & Cirelli, 2006) synaptic downscaling during sleep would result in reduced energy consumption. Thus, this reduced energy demands should be reflected in the oxygen metabolism and StO_2. Thirteen nights of 7 male subjects (age 11 - 16 years, 1 subject contributed only one night, all others two) were included in the analysis. We performed NIRS measurements throughout the entire night. The NIRS sensor was placed close to electrode position Fp1, (international 10/20 system) over the left frontal cortex. Absolute StO_2 and total haemoglobin (tHb) were calculated from the NIRS measurements using a self-calibrating method (Hueber et al., 1999). StO_2 and tHb during the awake period prior to sleep and after awakening were compared. The subjects were instructed to lie in bed in the same position before and after sleep. Values of the two nights were averaged for each subject. Furthermore, a linear regression line was fit through the all-night StO_2 recordings. We found an increase in StO_2 by 4.32 ± 1.76 % (mean ± SD, paired t-test $p < 0.001$, $N = 7$) in the morning compared to evening, while tHb did not change (1.02 ± 6.81 µM, $p = 0.704$, $N = 7$). Since the tHb remained at a similar level after sleep, this increase in StO_2 indicates that in the morning more oxygenated blood and less deoxygenated blood was present in the brain compared to the evening. The slope of the regression line was 0.37 ± 0.13 % h^{-1} leading to a similar increase of StO_2 in the course of sleep. This may be interpreted as a reduced oxygen consumption or energy metabolism after sleep.

4.4.1. Introduction

How does the oxygen metabolism change in the course of sleep? While different studies investigated specific events during sleep, e.g., sleep apnoea (Pizza, Biallas, Wolf, Werth, & Bassetti, 2010), differences between sleep stages (Näsi et al., 2011) or wakefulness and sleep (Spielman et al., 2000) the temporal evolution of the oxygen metabolism in the course of sleep is not clear. According to the synaptic homeostasis hypothesis (Tononi & Cirelli, 2006), a popular hypothesis about the function of sleep, oxygen demand should be reduced during sleep and hence brain metabolism should be decreased. A PET study (Braun et al., 1997) showed that global cerebral blood flow (CBF) after sleep was reduced compared to measurement before sleep onset. In an ultrasound doppler study in 6 healthy young males, blood flow velocity in the middle cerebral artery decreased by 6.6 % between pre- and post-sleep measurements (Kuboyama et al., 1997). Global CBF measured with PET could be a marker for oxygen consumption (Maquet, 2000) and in that sense the reduced global CBF may indicate a reduced energy consumption. However, this relation is not yet established and in order to obtain absolute CBF values arterial blood sampling is required. This makes PET rather impractical for sleep studies, in particular in children, which will also not be able to sleep in one position throughout the entire night. Near-infrared spectroscopy (NIRS) provides a non-invasive way to indirectly measure the oxygen metabolism which is related to the cerebral tissue oxygen saturation via the link of cytochrome C oxidase (Tachtsidis et al., 2009). Because of its non-invasive nature and the small size of the sensor, the subjects are free to move during sleep and the discomfort is reduced to a minimum. Hence NIRS may be an interesting tool to investigate the oxygen metabolism in sleep. The aim of the current study was to investigate sleep related changes in StO_2 by comparing StO_2 before and after sleep. The synaptic homeostasis hypothesis (Tononi & Cirelli, 2006) proposes a reduced energy consumption after sleep compared to before sleep, which should be reflected in StO_2.

4.4.2. Methods

Subjects

Data of seven healthy subjects (age 11 - 16 years, mean 13.6 years, all male) were recorded and analysed. Each subject spent two nights in the sleep laboratory at the Children's Hospital Zurich, separated by 3 weeks. Time in bed was approximately 8.5 h. Before the recordings in the sleep laboratory, the subjects had to keep a regular sleep-wake rhythm for at least 3 nights. The subjects had to fill in a sleep questionnaire and wore an activity monitor at the wrist of the non-dominant arm. This allowed us to check compliance with the instructions of keeping a regular sleep-wake

rhythm. The last 3 days before the night they were not allowed to consume caffeine containing products. The study was approved by the ethical committee of the Canton of Zurich and informed consent was obtained from the legal representatives.

Protocol

The near-infrared spectroscopy (NIRS) sensor was placed at the left forehead, close to the position of electrode Fp1, international 10/20 system (Jasper, 1958). Additionally to the NIRS measurement, high-density EEG recordings (128 electrode EEG net, Electrical Geodesics, Inc.) were performed during the night. EEG data are not presented here. In addition to the continuous measurements during the night we measured two minutes prior to light out and two minutes after awakening. We instructed the subject to lie on his back, looking at the ceiling and not to move. With an accelerometer (ADXL330, Analog Devices) built into the sensor we were able to exclude errors resulting from wrong head positions or head movements.

NIRS measurement

NIRS measurements were performed with an in-house built NIRS device, the *OxyPrem*, which is similar to previous wireless sensors (Muehlemann et al., 2008). It measures light attenuation at 760 nm, 805 nm and 870 nm, at the distances 1.5 cm and 2.5 cm. With this sensor we were able to calculate StO_2 for two different regions using the multi-distance method (Hueber et al., 1999). Region one is covering an area of approximately 3 cm^2 and was closer to electrode F3 (just below the hairline for most subjects). Region two was covering the same area close to electrode Fp1. Only data of region one are reported.

Post processing

To calculate StO_2 we use the simplified diffusion constant and did not account for water in tissue, as described in assumption A4 in (Metz, Biallas et al., 2013). This approach is based on the diffusion equation for a semi-infinite medium and a point-source and implies the assumption $r(3\mu_a\mu_s') >> 1$. Here r denotes the distance between source and detector, μ_a and μ_s' are the absorption and the reduced scattering coefficients, respectively.

For the scattering coefficients of the brain we used the values published by Matcher et al. (Matcher, Cope, & Delpy, 1997) and thus, were able to obtain total (tHb), oxygenated (O_2Hb) and deoxygenated haemoglobin (HHb). The relative change in tHb may be an indicator for the change in blood volume. The relation between StO_2 and tHb is given by: $StO_2 = O_2Hb / tHB$ and $tHb = O_2Hb + HHb$. To exclude errors in StO_2 resulting from unintended movement of the subject the accelerometer data were checked. By visual inspection, only those parts were included in the analysis with values around - 0.3 g and - 0.7 g in the y- and z-axis of the accelerometer, respectively. In this position the subject was lying on the back. The constant g represents the earth's gravity ($\approx 9.81\ m \cdot s^{-2}$). The included parts per measurement were averaged to

obtain one value in StO_2 and tHb for statistical analysis. To estimate the change of StO_2 over the night we calculated a linear regression and investigate the slope (% / h; see Fig. 4.4.2). Sleep stages were visually scored according to standard criteria.

Statistics

For statistical analysis we averaged the two nights per subject, leading to 7 evening–morning StO_2 and tHb pairs. These were compared by paired t-tests. All processing was performed by Matlab® (R2009b and R2011b, The Mathworks®, Natick, Massachusetts, USA).

4.4.3. Results

We found a significant increase in StO_2 of 4.32 ± 1.76 % (mean ± SD, $p < 0.001$, $N = 7$) in the morning compared to the evening. Mean StO_2 prior to sleep was 69.43 ± 2.02 % and 73.76 ± 2.36 % after awakening. The mean change in tHb was 1.02 ± 6.81 µM ($p = 0.704$, $N = 7$), which was not significant. Individual data are shown in Fig. 4.4.1. Furthermore the O_2Hb increased by 4.27 ± 4.46 µM ($p < 0.05$, $N = 7$) and HHb decreased by 3.25 ± 3.01 µM ($p < 0.05$, $N = 7$). The mean linear increase over the night was 0.37 ± 0.13 % h^{-1} (Fig. 4.4.1 and 4.4.2). The percentage increase indicates an absolute rather than a relative increase with respect to the baseline value.

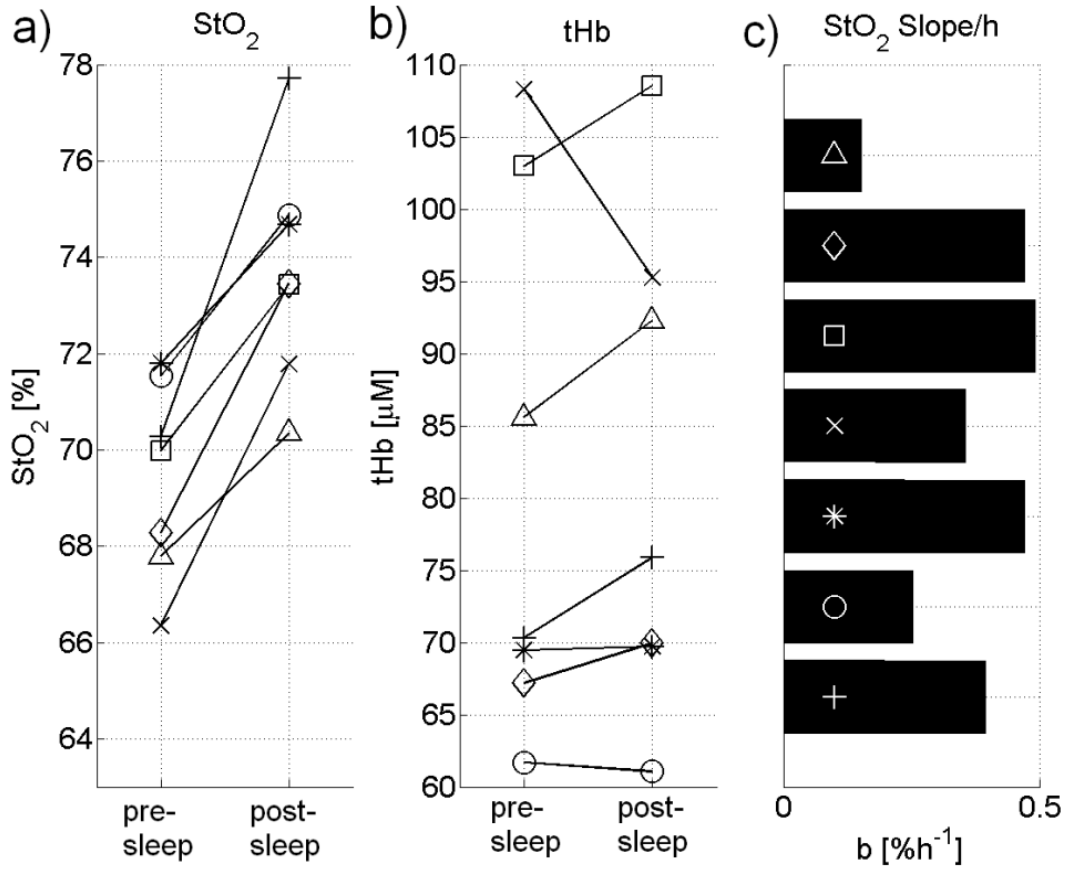

Figure 4.4.1. a) Brain tissue oxygen saturation (StO_2) increased in the morning after sleep (post-sleep), compared to the evening before sleep (pre-sleep). The values of the two nights were averaged except for subject (+) which only contributed with one night. b) Change of total haemoglobin (tHb) from evening to morning. On average no change was observed. c) Slope of the linear regression for StO_2 for the whole night (see Fig. 4.4.2). A positive slope was observed in all subjects and all nights. The different symbols indicate the 7 subjects.

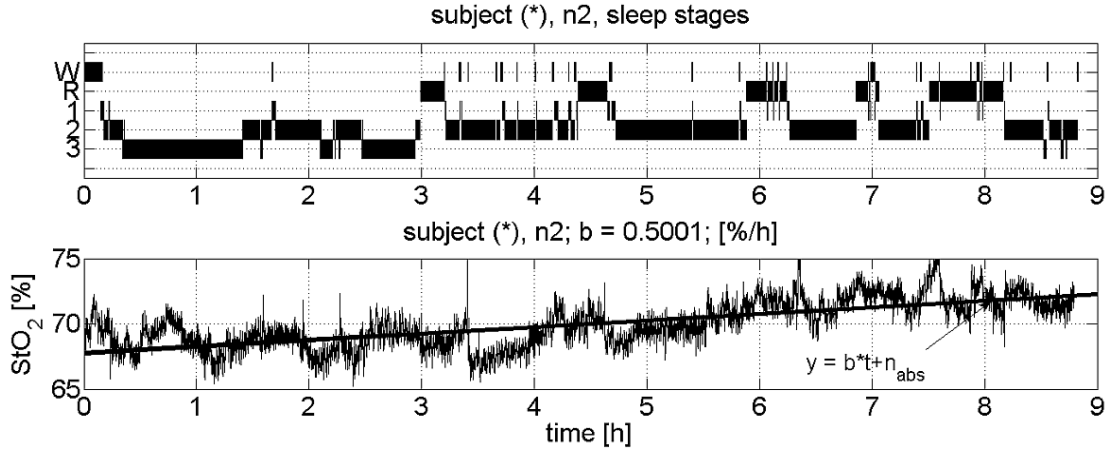

Figure 4.4.2. Top: Visually scored sleep stages for one night of subject (*) (W: waking; R: REM sleep; 1 to 3: non-REM sleep stages N1-N3). Bottom: Corresponding time course of StO_2. A linear regression line was fitted through the data. The slope (b) of the increase during the night is indicated at the top of the panel. Please note that the b value here is given for the individual night, while the b values in Fig. 4.4.1 are averaged over two nights.

4.4.4. Discussions and conclusion

In adolescent subjects we observed an increase in StO_2 in wakefulness post sleep compared to wakefulness pre sleep. This increase was confirmed when fitting a regression line through all-night StO_2 measurements. Since tHb did not change overnight, this increase in StO_2 may indicate that in the morning more oxygenated and less deoxygenated blood was present in the brain compared to the evening. The oxygen metabolic rate can be expressed a function of CBF, arterial oxygen saturation (SaO_2) and the cerebral tissue oxygen saturation (Elwell et al., 2005) and therefore our finding might be interpreted as a reduced oxygen consumption and thus, energy metabolism (linked by the oxidative phosphorylation, (Nelson, Lehninger, & Cox, 2008), Chap. 19) after sleep. This interpretation requires SaO_2 and the CBF to be constant. Since tHb overall remains constant, the Cerebral blood volume (CBV) remains constant and hence does the CBF, which is related to the CBV (Grubb et al., 1974). Since we did not measure the SaO_2 we cannot be sure whether this assumption holds, however the subjects were healthy and no sleep apnoeas were observed during the night, which makes the assumption more plausible. A reduced energy metabolism would be in line with the synaptic homeostasis hypothesis (Tononi & Cirelli, 2006), which states that synaptic downscaling during the night would lead to a reduced energy demand of the brain. Alternatively, the increased StO_2 could be linked to circadian effects (independent of sleep), as e.g., cortisol rhythm which exerts its wake promoting effect in the early morning hours (Cajochen, Chellappa, & Schmidt, 2010). As can be seen in Fig. 4.4.2 the assumed linear trend describes the StO_2 increase during sleep fairly accurate regarding the whole-night changes. But on a shorter time scale fluctuations were evident. At this point we speculate that these fluctuations are movement induced on the one hand and related to changes in sleep on the other.

In summary, in adolescents we found an increase in cerebral tissue oxygen saturation in the course of sleep, which may represent a reduced oxygen consumption of the brain and therefore a lower energy metabolism. Thus, our data are in line with the synaptic homeostasis hypothesis (Tononi & Cirelli, 2006).

5. Discussion

The overall aim of this work was to assess changes in neuronal activity during sleep by measuring oxygen consumption using NIRS and high-density (HD) EEG. Also, we pursued to detect changes in local SWA in relation to cognitive performance after intensive working memory training.

Thus, the first approach was to assess metabolic correlates of neuronal activity by combining NIRS with HD EEG. We were able to acquire NIRS signals over the whole night to measure oxygen tissue saturation as a correlate for cerebral oxygen consumption. In a next step, we pursued to investigate effects of working memory training on cognitive performance and on sleep SWA. We found that the intensive working memory training induced performance increases not only immediately after the training, but also long-term. Furthermore, the training may have induced regional synaptic modifications as shown by local changes in sleep SWA. At last, we found that StO_2 increased during NREM sleep overnight. This finding will be discussed in relation to a functional role of slow waves as proposed by the synaptic homeostasis hypothesis.

Hemodynamic changes between vigilance states measured by NIRS

In a first step, we assessed oxygen consumption at the transition of vigilance states, where changes in energy consumption are to be expected. As presented in section 4.1, we observed hemodynamic changes at vigilance state transitions. They were comparable to changes reported in adults (e.g., Näsi et al., 2011). By trend, [O_2Hb], [HHb] and [tHb] showed time courses in line with the expectations. Specifically, the changes in neuronal activity from sleep stages with less neuronal activity (or lower firing rate), i.e. NREM sleep, to sleep stages with higher firing rate, i.e. REM sleep, [O_2Hb] and [tHb] increased whereas [HHb] decreased. The opposite effect was observed for the transition of REM to NREM sleep stages and at sleep onset. This finding supports the assumption that effects of neurovascular coupling associated with changes in oxygen consumption (and hence related to neuronal activity) can be observed by NIRS. Not all of the NIRS measures were statistically significant though. Particularly, [O_2Hb] shows a high standard deviation (consequently also reflected in [tHb]). StO_2 which was introduced in this work seems to provide a more stable measure for assessing overnight changes in neuronal activity. StO_2 changes were significant for all observed vigilance state transitions: it decreased at sleep onset and from REM to NREM sleep, and increased from NREM to REM sleep. For future studies, one should eliminate the possibility that changes of arterial oxygenation are the underlying cause for the observed StO_2 variations, e.g., due to variations in systemic oxygen turnover. This could be achieved by concurrently measuring arterial oxygen saturation using pulse oximetry which helps to distinguish between localized

effects (hemodynamic changes in the cortex) and systemic effects (e.g., blood pressure changes).

Working memory training

Our second hypothesis was that intensive working memory training may stimulate working memory performance effectively if long-term effects were observable. Furthermore, we tested if transfer effects to untrained cognitive tasks can be observed. We found that the visuospatial n-back training induced an increase in auditory n-back performance immediately and some months after the training. On cognitive level, as discussed in sections 4.2 and 4.3, the long-term effects observed in the auditory n-back task performance may be indicative for the efficacy of such a training, since a decrease at follow-up (FU) back to the baseline performance or to the performance level of the control group would suggest a mere practice effect. Long-term effects in relation to the changes in local sleep SWA are further discussed below.

According to our hypothesis, beneficial effects of working memory training may be substantiated if also cognitive tasks other than working memory profited from the training. However, in contrast to other studies (Jaeggi et al., 2008; Jaeggi, Buschkuehl et al., 2011), fluid intelligence measured with a nonverbal matrix reasoning task was not significantly affected by the training (mixed-model ANOVA). Only within the training group, performance was significantly increased at FU compared to PRE, with the number of subjects being one limiting factor. The limited success when enrolling subjects may have been caused by the dense study protocol which discouraged interested candidates. For future training studies, performing a power analysis based on our data will be beneficial.

However, it is a matter of debate if working memory may lead to transfer effects to untrained tasked at all (Owen et al., 2010; Redick et al., 2013; Shipstead, Redick, & Engle, 2012). A recently published meta-analysis revealed that working memory training only lead to short-term improvements in the trained task only (Melby-Lervåg & Hulme, 2013). Thus, although working memory capacity supports higher cognitive functions including reasoning (Colom, Rebollo, Palacios, Juan-Espinosa, & Kyllonen, 2004), the effects of working memory on general intelligence and other cognitive tasks are controversially discussed as reports of effects on more distant, untrained cognitive functions are diverging. For example, Redick et al. performed a carefully designed study, being aware of many aspects such as the inclusion of an active control group and the assessment of several cognitive tasks before, within and after the training period; also they reached high statistical power (Redick et al., 2013). They did not find performance changes in any of the untrained tasks, including fluid intelligence or perceptual speed. Thus, to this point, it is unclear if, or under which conditions, working memory training transfers to fluid intelligence and other untrained cognitive functions. As suggested by Redick et al., further effort should be

made to inspect training performance in order to overcome the controversy regarding the results on cognitive performance (Redick et al., 2013).

In our study, we assessed working memory training amount and performance. This revealed that auditory n-back performance increase did not correlate with training amount but rather seemed to depend on training performance: for those subjects who steadily increased their working memory training performance during the training period, effects of the training on auditory n-back performance were larger than for the unsteady training group. However, the steady performers were older by trend and achieved higher fluid intelligence scores at PRE than the unsteady performers. For future experiments, the inclusion of more subjects would help to disentangle the most important contributing factors (e.g., age, baseline IQ, training performance increase) which influence training-induced performance increases. For the applied visuospatial n-back task, older subjects (or those with more mature cognition) may have been more disciplined because for them the rather abstract training task and the goal of the training were potentially more comprehensible than for younger subjects. For younger subjects on the other hand, a short-term reward should be considered in the study, subjects were given a gift only at POST). Thus, overall, our results presented in section 4.2 as well as other reports on working memory training offer still some caveats. However, with our third aim, pursuing to detect working memory training-induced changes in SWA, a new approach is offered to assess the effectiveness of cognitive training.

Local changes in SWA after working memory training

In the third study, we aimed to investigate working memory training-induced synaptic plasticity by measuring SWA with HD EEG. As discussed above, we suggest that our applied working memory training was successful in inducing cognitive plasticity since it induced increase in auditory n-back performance immediately as well as long-term. Our aim was to use SWA topography as a tool to map these training-induced synaptic changes. As presented in section 4.3, SWA was locally increased after three weeks of visuospatial n-back training in a region that is known to be involved in working memory performance. Furthermore, auditory n-back performance at POST and FU correlated positively with the increase in SWA in this region, located over the left frontoparietal cortex (*cluster T*, see Fig. 4.3.2 in section 4.3).

To our knowledge, this is the first study reporting local changes in SWA after an intensive cognitive training period in young healthy subjects. The local increase in SWA may reflect synaptic plastic changes that were induced by the training (see discussion in section 4.3).

As introduced in section 3.2.3, spontaneous wake and learning are associated with LTP-induced synaptic potentiation and plasticity (Bushey et al., 2011; Vyazovskiy et al., 2008). The increased synaptic connections in turn determine synchrony and am-

plitude of slow waves (Esser et al., 2007; Riedner et al., 2007; Vyazovskiy et al., 2007). Thus, we suggest that the strengthening of synaptic connections in *cluster T* during wake leads to an increase of synchronous and high-amplitude slow waves at night after three weeks of working memory training. In line with other studies, also our results indicate that learning induces synaptic plasticity as reflected by the local SWA increase. Further, these synaptic plastic changes in the left frontoparietal area may contribute to the enhanced performance in auditory n-back some months after the training. These local synaptic connections may not only have been functionally modified, but also structurally, e.g., by an increase in spine number and density. Overall, our results emphasize the importance of intervention studies using learning paradigms to manipulate synaptic strength and to determine the causal relationship between learning, synaptic plasticity and changes in SWA.

There are studies indicating that slow waves not only reflect, but also contribute to performance changes during wake. For example in cats, Chauvette et al. showed that mimicking slow waves by electrical stimulation seemed to induce LTP (Chauvette, Seigneur, & Timofeev, 2012). Also, as discussed in the introduction (see section 3.2.3), the synaptic homeostasis hypothesis suggests that slow waves improve the signal-to-noise ratio by downscaling synaptic strength (Olcese et al., 2010). This implies that the strongest connections, e.g., strengthened by a learning task, prevail as opposed to weaker connections. Thus, both mechanisms LTP as well as LTD-like processes in *cluster T* may influence long-term performance in auditory n-back performance at FU.

Regarding the possible contribution of slow waves to synaptic plasticity and thereby performance, a third measurement of sleep SWA at FU would have been interesting in order to further elucidate the relationship between synaptic plasticity and the role of slow waves after having finished working memory training. We did not include such a third overnight sleep recording because of the already very dense study protocol. Thus, it remains open if the increase in SWA in *cluster T* would have remained stable, in line with the sustained performance increase in auditory n-back performance, or if it would have reached its maximum at POST or even earlier.

A sustained increase in SWA at follow-up may be explained by ‘down-selection’, a process which protects strong synaptic connections from depression (Nere, Hashmi, Cirelli, & Tononi, 2013). However, as shown by Kurth et al. (Kurth et al., 2010), peak SWA shifts during development and thus, together with experience-induced changes over time, *cluster T* may vanish after some months. Furthermore, it should be considered that in section 4.2, we showed that mean maximal training performance was reached at 60 % of the training period and that this maximal performance was significantly higher than performance at the last day of training (i.e. at POST). However, to capture the moment of maximal training performance is highly challenging: it would ask for a spontaneously organized sleep EEG measurement at this

night, besides having a reasonable method to detect the moment when training performance starts to decrease or stagnate.

The downscaling processes during sleep discussed above may be functionally relevant for energy restoration (Tononi & Cirelli, 2006). As shown in section 4.1, NIRS seems to be applicable to measure oxygen consumption during the night. Thus, our fourth aim was to investigate energy consumption of the brain during sleep.

Hemodynamic changes over night measured by NIRS

To assess energy consumption, StO_2 was measured over the course of the entire night. Linear regression of the StO_2 value revealed an overnight increase (see Fig. 4.4.1 in section 4.4). Meanwhile, [tHb] seemed to remain constant overnight, which indicates that the cerebral blood volume and thus, cerebral perfusion did not change. Therefore, we attributed the increase in StO_2 not to an increased oxygen supply but to a reduction in [HHb] and thus, a reduction of metabolic turnover: the StO_2 value raises with decreasing oxygen demand of the neurons (and vice versa). The overnight decrease in metabolic turnover is consistent with the synaptic homeostasis hypothesis (Tononi & Cirelli, 2006). The synaptic homeostasis hypothesis claims that synaptic strength decreases overnight by downscaling. This is reflected by a decrease in SWA overnight (Achermann & Borbély, 2011) as shown in Fig. 5.1 as an example of one subject.

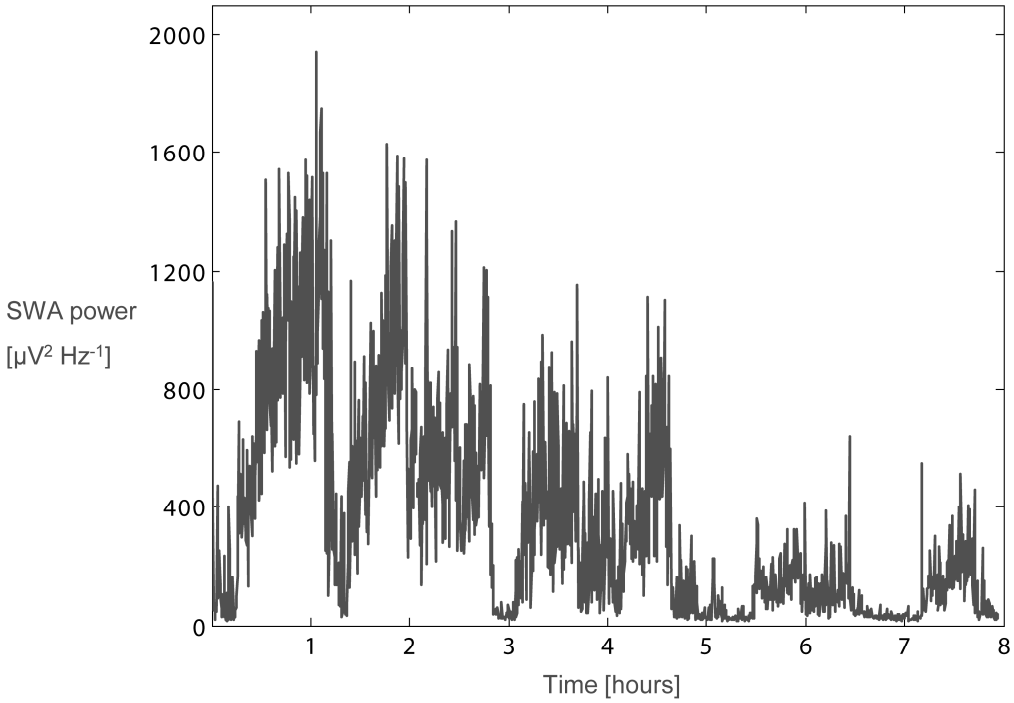

Figure 5.1: Slow wave activity (SWA, 1 to 4.5 Hz) from lights-off to lights-on, depicted for one subject, decreases over the night.

Thus, after a sleep episode, synaptic strength is downscaled to make energy available again for further synaptic strengthening during the following wake episode. This interpretation is in line with the increase in cortical glucose concentrations during NREM sleep which was reported in literature (Dash, Bellesi, Tononi, & Cirelli, 2013). With respect to learning and memory, this means that the brain is being prepared during sleep to acquire and process new information during daytime. Thus, the amount of StO_2 increase overnight as measured by NIRS might serve as an indicator for synaptic downscaling and for learning capacity during wake.

However, the proposed link between StO_2 and the synaptic homeostasis hypothesis remains descriptive. An attempt to correlate StO_2 and SWA could not strengthen the above findings because Metz and colleagues did not find a reliable correlation between the two variables (unpublished). It was proposed to eliminate the influence of circadian effects (process C), e.g., by performing sleep deprivation experiments. This idea was followed up with a single-subject pilot measurement, which revealed that during prolonged wakefulness, StO_2 did not increase as shown in section 4.4 and neither seemed to be homeostatically regulated, since it did not increase stronger in the recovery night. One factor that influences StO_2 may be body temperature (Krauchi & Wirz-Justice, 1994), which was shown to follow a circadian rhythm. Vasodilation and vasoconstriction, basic mechanisms for thermoregulation (Benzinger, 1969), may influence StO_2 concentrations. However, this finding should be considered with caution, as it was only shown in one subject. Furthermore, the experiment was not performed under controlled conditions to eliminate the influence of exogenous parameters, e.g., food intake, and constant body posture (e.g., Duffy & Dijk, 2002), which could be achieved by constant routine protocol.

Concluding remarks

We showed that measuring oxygen consumption with NIRS during sleep offers a novel possibility to extend EEG investigations of neuronal activity and synaptic plasticity. With the local increase in SWA, in a region known to be important for working memory, a new measure to assess training effectiveness was presented, since investigating sleep SWA changes after training is suggested to reflect training-induced synaptic plasticity. Thus, working memory training may serve to manipulate synaptic strength and, taking the long-term effects of the training on cognitive performance into account, it seems to be a suitable tool for investigating learning processes over several days and weeks.

As mentioned earlier, the number of subjects was rather low considering the broad age range. We targeted a young population because we expected major plastic changes due to the ongoing maturational processes. However, at this age range, cognitive performance (Luna et al., 2004) as well as SWA (Feinberg et al., 2006) are under development and are thus, highly heterogeneous. A higher number of study sub-

jects would help to mitigate this challenge. In addition, to maintain an active control group should be considered in order to control for non-specific effects of the training. Also, for future investigations in the field we would consider a more attractive training task, appropriate for the specific age range. Although we had some means to verify the training compliance of the subjects (retrospectively as well as through one home visit which was conducted within the training period), surveillance of the training compliance may be improved, e.g., by providing the training software on an internet-based server which can be surveyed by the investigators. In summary, increasing the number of subjects within a narrower age range as well as applying some modifications to the training task may help to elucidate effectiveness of working memory training.

Yet despite these caveats, a pilot study conducted on subjects with working memory deficits seems to be promising with regard to the efficacy of working memory training. Poor working memory performance was shown to negatively affect many aspects of cognitive processes, including educational achievements in skills such as reading (Wang & Gathercole, 2013) and mathematics (Passolunghi & Mammarella, 2012). Considering these aspects as well as our observation of a beneficial effect of visuospatial n-back training on auditory working memory in a normal population, we extended our protocol to a population of young subjects with working memory deficits. For this pilot study we included subjects between 8 and 16 years of age who had consulted the Child Development Center (University Children's Hospital Zurich) because they had experienced problems at school. A main inclusion criterion was normal overall IQ but low working memory performance as assessed by HAWIK (Hamburg-Wechsler-Intelligenztest für Kinder) subtests including letter-number sequencing (LNS) task. Preliminary results in five subjects revealed a significant performance increase in LNS immediately after the visuospatial n-back training (see Fig. 5.2), which is the same training as performed by our non-clinical group. Similar as our non-clinical group, the subjects of the pilot study also improved in auditory n-back performance short- and long-term. By including more subjects we expect to be able to answer the question if working memory training may indeed mitigate deficits in working memory performance. Further, also in this population sleep HD EEG may be applied with the aim to detect plasticity-induced changes in SWA.

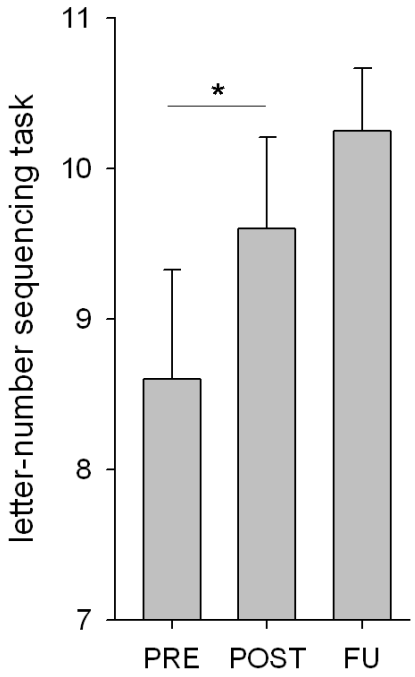

Figure 5.2: Performance in letter-number sequencing task (LNS, mean ± SEM) for subjects with diagnosed working memory deficit. PRE: before training (N = 5), POST: immediately after three weeks of working memory training (N = 5), FU: Follow-up after approximately 2.5 months (N = 4). * indicates *p*-value < 0.05 (paired t-test).

The study presented in this dissertation is the first to show a local increase in SWA after an intensive cognitive training over three weeks, indicating that synaptic plasticity was induced. However, our results remain correlative. For instance, we do not know if *cluster T* is specific for auditory working memory. To further substantiate a relationship between synaptic plasticity and SWA, the study protocol could be enhanced, e.g., by direct manipulation of synaptic strength by TMS or by enhancing the contribution of NIRS.

TMS application was shown to change cortical excitability and slow waves (for review, see Massimini, Tononi, & Huber, 2009). Applying TMS during wake induced changes in subsequent sleep SWA in the stimulated region (Huber et al., 2008). Furthermore, behavioral measures such as decision making were changed due to TMS application over specific cortical regions related to the behavioral task (Cho et al., 2012; Knoch et al., 2006; Ott, Ullsperger, Jocham, Neumann, & Klein, 2011). Regarding these studies, the relevance of *cluster T* for working memory may be validated by using TMS. Repetitive and high frequent stimulation of the cortex in *cluster T* by TMS may increase cortical excitability and strengthen synaptic connections in this area. If *cluster T* indeed reflects synaptic plastic changes due to training, and if it is important for working memory performance, then TMS stimulation could have a comparable effect as the intensive training. Specifically, a third group of subjects performing no training, but receiving TMS, may show a comparable increase in auditory n-back performance and SWA as the training group. Other techniques to directly

manipulate slow waves and consequently behavioral measures include electrical (Marshall, Helgadottir, Molle, & Born, 2006) or auditory stimulation during slow wave sleep (Ngo, Martinetz, Born, & Molle, 2013).

For future studies which investigate learning and changes in SWA, NIRS may be applied as well. As discussed above, such measurements would profit from some adaptations, e.g., by adding pulse oximetry in order to discriminate between systemic and localized effects. Furthermore, using a NIRS device with more light-sources and detectors, as did for example Uchita-Ota et al. (Uchida-Ota et al., 2008), or by positioning our in-house built sensor over the cortical areas of *cluster T* may extend the explanatory power of NIRS when applied as a supplement to HD EEG. During sleep however, a more posterior location of the sensor over *cluster T* would increase the problem of movement artifacts. Alternatively, hemodynamic changes could be measured while executing a working memory task before and after the training. If synaptic plasticity has been induced by the training, then the strengthened synaptic connections would not only be reflected in an increase in SWA after the training, but might also be measurable as changes in tissue oxygen saturation while the task is performed. Specifically, StO_2 would increase from PRE to POST.

Overall, we could confirm that HD EEG serves as an easily applicable and inexpensive tool (Lustenberger & Huber, 2012), which is well accepted by the subjects. For the first time, we accomplished to implement NIRS into HD EEG in a young population. Our finding indicates that StO_2 may serve as a measure to follow up on the questions addressing the functional role of sleep, and especially sleep slow waves, in relation to energy restoration and synaptic homeostasis. We could also show that working memory training over three weeks, despite the need of improvements concerning e.g., its attractiveness, may be applied in young subjects, for both a healthy as well as a clinical population. For future studies, the observed long-term effects of the training on cognitive performance implicate that training effects should also be studied after several months. The local increase in SWA after the working memory training presented here remains correlative to some extent. However, our findings contribute to the evidence that SWA may be used as a tool to map learning-induced plasticity not only overnight, but also after three weeks of cognitive training.

6. Terms and abbreviations

[HHb]	Deoxyhemoglobin concentration
[O_2Hb]	Oxyhemoglobin concentration
[tHb]	Total hemoglobin concentration
μ_a	Absorption coefficient
μ_s	Reduced scattering coefficient
AASM	American academy of sleep medicine
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	Analysis of variance
BA	Brodmann area
BDNF	Brain-derived neurotrophic factor
BOLD	Blood oxygen level dependent
CaMKII	Ca^{2+}-calmodulin-dependent protein kinase II
CBF	Cerebral blood flow
CBV	Cerebral blood volume
CO_2	Carbon dioxide
DSS	Digit symbol search task
EEG, HD	Electroencephalography, high-density
E-LTP	Early long-term potentiation
EPSP	Excitatory postsynaptic potential
FD	Forced desynchrony
FDR	False discovery rate
FFT	Fast Fourier transformation
fMRI	Functional magnetic resonance imaging
FT	Flanker task
FU	Follow-up
GABA	Gamma-aminobutyric acid
GluR	Glutamate receptor
HAWIK	Hamburg-Wechsler-Intelligenztest für Kinder
IPSP	Inhibitory postsynaptic potential
IQ	Intelligence quotient
L-LTP	Late long-term potentiation
LNS	Letter-number sequencing
LTD	Long-term depression

LTP	Long-term potentiation
MAD	Median absolute deviation
MAT	Matrix reasoning task
mEPSC	Miniature excitatory postsynaptic current
MRI	Magnetic resonance imaging
N	Number of subjects
n	Load factor in n-back task
N1, N2, N3	NREM sleep stage 1, 2, 3
NIRS	Near-infrared spectroscopy
NMDA	*N*-methyl-$_D$-aspartate
NREM	Non-rapid eye movement
NST	Number-span task
PET	Positron emission tomography
PPT-LDT	Pedunculopontine tegmental - laterodorsal tegmental nuclei
PSP	Postsynaptic potential
RE	Thalamic reticular
REM	Rapid eye movement
RT	Reaction time
SaO_2	Arterial oxygen saturation
SCN	Suprachiasmatic nucleus
SEM	Standard error of the mean
SnPM	Statistical non-parametric mapping
SST	Symbol search task
ST	Stroop task
STDP	Spike timing dependent plasticity
StO_2	Tissue oxygen saturation
SWA	Slow wave activity
TC	Thalamocortical
TMN	tuberomammillary nucleus
TMS	Transcranial magnetic stimulation
TONI-IV	Test of non-verbal intelligence - version IV
VNBT	Visual n-back training

7. References

Achermann, P. (2009). EEG analysis applied to sleep. *Epileptologie, 26,* 28-33.

Achermann, P., & Borbély, A. A. (2011). Sleep homeostasis and models of sleep regulation. In M. H. Kryger, T. Roth & W. C. Dement (Eds.), *Principles and Practice of Sleep Medicine* (5th ed., pp. 405-417). Philadelphia: Elsevier Saunders.

Agresti, A., & Finlay, B. (2008). *Statistical Methods for the Social Sciences* (4th ed.).

Alloway, T. P., Gathercole, S. E., Kirkwood, H., & Elliott, J. (2009). The cognitive and behavioral characteristics of children with low working memory. *Child Dev, 80*(2), 606-621.

Amzica, F., & Steriade, M. (1997). The K-complex: its slow (<1-Hz) rhythmicity and relation to delta waves. *Neurology, 49*(4), 952-959.

Attwell, D., & Laughlin, S. B. (2001). An energy budget for signaling in the grey matter of the brain. *Journal of Cerebral Blood Flow and Metabolism, 21*(10), 1133-1145.

Baddeley, A. (2000). The episodic buffer: a new component of working memory? *Trends Cogn Sci, 4*(11), 417-423.

Baddeley, A. (2003). Working memory: looking back and looking forward. *Nat Rev Neurosci, 4*(10), 829-839.

Baddeley, A., & Hitch, G. J. (Eds.). (1974). *Working Memory* (Vol. 8). New York: Academic Press.

Bailey, C. H., & Kandel, E. R. (2008). Synaptic remodeling, synaptic growth and the storage of long-term memory in Aplysia. *Prog Brain Res, 169,* 179-198.

Bäumle, G. (1985). *Farbe-Wort-Interferenztest nach J.R. Stroop (FWIT)*. Göttingen: Hogrefe.

Bear, M. F. (1995). Mechanism for a sliding synaptic modification threshold. *Neuron, 15*(1), 1-4.

Bear, M. F., & Malenka, R. C. (1994). Synaptic plasticity: LTP and LTD. *Curr Opin Neurobiol, 4*(3), 389-399.

Benington, J. H., & Frank, M. G. (2003). Cellular and molecular connections between sleep and synaptic plasticity. *Prog Neurobiol, 69*(2), 71-101.

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate - a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B-Methodological, 57*(1), 289-300.

Benke, T. A., Luthi, A., Isaac, J. T., & Collingridge, G. L. (1998). Modulation of AMPA receptor unitary conductance by synaptic activity. *Nature, 393*(6687), 793-797.

Benzinger, T. H. (1969). Heat regulation: homeostasis of central temperature in man. *Physiological Reviews, 49*(4), 671-759.

Blomqvist, G., Seitz, R. J., Sjogren, I., Halldin, C., Stone-Elander, S., Widen, L. (1994). Regional cerebral oxidative and total glucose consumption during rest and activation studied with positron emission tomography. *Acta Physiol Scand, 151*(1), 29-43.

Borbely, A. A. (1982). A two process model of sleep regulation. *Hum Neurobiol, 1*(3), 195-204.

Borbély, A. A., Baumann, F., Brandeis, D., Strauch, I., & Lehmann, D. (1981). Sleep deprivation: effect on sleep stages and EEG power density in man. *Electroencephalography and Clinical Neurophysiology, 51*(5), 483-495.

Braun, A. R., Balkin, T. J., Wesenten, N. J., Carson, R. E., Varga, M., Baldwin, P. (1997). Regional cerebral blood flow throughout the sleep-wake cycle. An H2(15)O PET study. *Brain, 120 (Pt 7)*, 1173-1197.

Brown, L., Sherbenou, R., & Johnsen, S. K. (1997). *Test of Nonverbal Intelligence, Third Edition* (Vol. 3rd). Austin, TX: PRO-ED.

Buchmann, A., Kurth, S., Ringli, M., Geiger, A., Jenni, O. G., & Huber, R. (2011). Anatomical markers of sleep slow wave activity derived from structural magnetic resonance images. *J Sleep Res, 20*(4), 506-513.

Buchmann, A., Ringli, M., Kurth, S., Schaerer, M., Geiger, A., Jenni, O. G. (2011). EEG Sleep Slow-Wave Activity as a Mirror of Cortical Maturation. *Cerebral Cortex, 21*(3), 607-615.

Bull, R., Espy, K. A., & Wiebe, S. A. (2008). Short-term memory, working memory, and executive functioning in preschoolers: longitudinal predictors of mathematical achievement at age 7 years. *Dev Neuropsychol, 33*(3), 205-228.

Buonomano, D. V., & Merzenich, M. M. (1998). Cortical plasticity: from synapses to maps. *Annu Rev Neurosci, 21*, 149-186.

Buschkuehl, M., Jaeggi, S. M., Hutchison, S., Perrig-Chiello, P., Dapp, C., Muller, M. (2008). Impact of working memory training on memory performance in old-old adults. *Psychol Aging, 23*(4), 743-753.

Buschkuehl, M., Jaeggi, S. M., Kobel, A., & Perrig, W. J. (2007). *BrainTwister - A collection of cognitive training tasks* (1.0.2 ed.). Bern.

Bushey, D., Tononi, G., & Cirelli, C. (2011). Sleep and synaptic homeostasis: structural evidence in Drosophila. *Science, 332*(6037), 1576-1581.

Cajochen, C., Chellappa, S., & Schmidt, C. (2010). What keeps us awake? The role of clocks and hourglasses, light, and melatonin. *International Review of Neurobiology, 93*, 57-90.

Cajochen, C., Foy, R., & Dijk, D. J. (1999). Frontal predominance of a relative increase in sleep delta and theta EEG activity after sleep loss in humans. *Sleep Res Online, 2*(3), 65-69.

Cajochen, C., Khalsa, S. B., Wyatt, J. K., Czeisler, C. A., & Dijk, D. J. (1999). EEG and ocular correlates of circadian melatonin phase and human performance decrements during sleep loss. *Am J Physiol, 277*(3 Pt 2), R640-649.

Campbell, I. G., & Feinberg, I. (2009). Longitudinal trajectories of non-rapid eye movement delta and theta EEG as indicators of adolescent brain maturation. *Proc Natl Acad Sci U S A, 106*(13), 5177-5180.

Caroni, P., Donato, F., & Muller, D. (2012). Structural plasticity upon learning: regulation and functions. *Nat Rev Neurosci, 13*(7), 478-490.

Carskadon, M. A., & Dement, W. C. (2011). Normal Human Sleep: An Overview *Principles and Practice of Sleep Medicine* (5th ed., pp. 16-26): Elsevier Saunders.

Chauvette, S., Seigneur, J., & Timofeev, I. (2012). Sleep oscillations in the thalamocortical system induce long-term neuronal plasticity. *Neuron, 75*(6), 1105-1113.

Chein, J. M., & Morrison, A. B. (2010). Expanding the mind's workspace: training and transfer effects with a complex working memory span task. *Psychon Bull Rev, 17*(2), 193-199.

Chetkovich, D. M., Gray, R., Johnston, D., & Sweatt, J. D. (1991). N-methyl-D-aspartate receptor activation increases cAMP levels and voltage-gated Ca2+ channel activity in area CA1 of hippocampus. *Proc Natl Acad Sci U S A, 88*(15), 6467-6471.

Cho, S. S., Pellecchia, G., Ko, J. H., Ray, N., Obeso, I., Houle, S. (2012). Effect of continuous theta burst stimulation of the right dorsolateral prefrontal cortex on cerebral blood flow changes during decision making. *Brain Stimul, 5*(2), 116-123.

Cirelli, C., & Tononi, G. (2000). Differential expression of plasticity-related genes in waking and sleep and their regulation by the noradrenergic system. *Journal of Neuroscience, 20*(24), 9187-9194.

Collingridge, G. L., Isaac, J. T., & Wang, Y. T. (2004). Receptor trafficking and synaptic plasticity. *Nat Rev Neurosci, 5*(12), 952-962.

Colom, R., Rebollo, I., Palacios, A., Juan-Espinosa, M., & Kyllonen, P. C. (2004). Working memory is (almost) perfectly predicted by g. *Intelligence, 32*(3), 277-296.

Contreras, D., & Steriade, M. (1995). Cellular basis of EEG slow rhythms: a study of dynamic corticothalamic relationships. *J Neurosci, 15*(1 Pt 2), 604-622.

Contreras, D., Timofeev, I., & Steriade, M. (1996). Mechanisms of long-lasting hyperpolarizations underlying slow sleep oscillations in cat corticothalamic networks. *J Physiol, 494 (Pt 1)*, 251-264.

Conway, A. R. A., Cowan, N., Bunting, M. F., Therriault, D. J., & Minkoff, S. R. B. (2002). A latent variable analysis of working memory capacity, short-term memory capacity, processing speed, and general fluid intelligence. *Intelligence, 30*(2), 163-183.

Costa-Mattioli, M., Sossin, W. S., Klann, E., & Sonenberg, N. (2009). Translational control of long-lasting synaptic plasticity and memory. *Neuron, 61*(1), 10-26.

Cueni, L., Canepari, M., Lujan, R., Emmenegger, Y., Watanabe, M., Bond, C. T. (2008). T-type Ca2+ channels, SK2 channels and SERCAs gate sleep-related oscillations in thalamic dendrites. *Nat Neurosci, 11*(6), 683-692.

Czeisler, C. A., Duffy, J. F., Shanahan, T. L., Brown, E. N., Mitchell, J. F., Rimmer, D. W. (1999). Stability, Precision, and Near-24-Hour Period of the Human Circadian Pacemaker. *Science, 284*(5423), 2177-2181.

Czeisler, C. A., & Gooley, J. J. (2007). Sleep and circadian rhythms in humans. *Cold Spring Harb Symp Quant Biol, 72*, 579-597.

Dahlin, E., Dahlin, L., Nyberg, L., Bäckman, A., & Neely. (2008). Plasticity of executive functioning in young and older adults: Immediate training gains, transfer, and long-term maintenance. *Psychology and aging, 23*(4), 720-730.

Dang-Vu, T. T. (2012). Neuronal oscillations in sleep: insights from functional neuroimaging. *Neuromolecular Med, 14*(3), 154-167.

Dang-Vu, T. T., Schabus, M., Desseilles, M., Sterpenich, V., Bonjean, M., & Maquet, P. (2010). Functional neuroimaging insights into the physiology of human sleep. *Sleep, 33*(12), 1589-1603.

Dash, M. B., Bellesi, M., Tononi, G., & Cirelli, C. (2013). Sleep/wake dependent changes in cortical glucose concentrations. *Journal of Neurochemistry, 124*(1), 79-89.

Delpy, D. T., Cope, M., Van der Zee, P., Wray, S., & Wyatt, J. (1988). Estimation of optical pathlength through tissue from direct time of flight measurement. *Physics in Medicine and Biology, 33*(12).

Diamond, A., & Lee, K. (2011). Interventions shown to aid executive function development in children 4 to 12 years old. *Science, 333*(6045), 959-964.

Dijk, D. J., & Czeisler, C. A. (1994). Paradoxical timing of the circadian rhythm of sleep propensity serves to consolidate sleep and wakefulness in humans. *Neurosci Lett, 166*(1), 63-68.

Dijk, D. J., & Czeisler, C. A. (1995). Contribution of the circadian pacemaker and the sleep homeostat to sleep propensity, sleep structure, electroencephalographic slow waves, and sleep spindle activity in humans. *J Neurosci, 15*(5 Pt 1), 3526-3538.

Dijk, D. J., Hayes, B., & Czeisler, C. A. (1993). Dynamics of electroencephalographic sleep spindles and slow wave activity in men: effect of sleep deprivation. *Brain Res, 626*(1-2), 190-199.

Duffy, J. F., & Dijk, D.-J. (2002). Getting Through to Circadian Oscillators: Why Use Constant Routines? *Journal of Biological Rhythms, 17*(1), 4-13.

Duncan, A., Meek, J. H., Clemence, M., Elwell, C. E., Fallon, P., Tyszczuk, L. (1996). Measurement of cranial optical path length as a function of age using phase resolved near infrared spectroscopy. *Pediatric Research, 39*(5), 889-894.

Elgersma, Y., & Silva, A. J. (1999). Molecular mechanisms of synaptic plasticity and memory. *Curr Opin Neurobiol, 9*(2), 209-213.

Elwell, C. E., Henty, J. R., Leung, T. S., Austin, T., Meek, J. H., Delpy, D. T. (2005). Measurement of CMRO2 in neonates undergoing intensive care using near infrared spectroscopy. *Advances in Experimental Medicine and Biology, 566*, 263-268.

Engle, R. W. (2002). Working Memory Capacity as Executive Attention. *Current Directions in Psychological Science, 11*(1), 19-23.

Esser, S. K., Hill, S. L., & Tononi, G. (2007). Sleep homeostasis and cortical synchronization: I. Modeling the effects of synaptic strength on sleep slow waves. *Sleep, 30*(12), 1617-1630.

Feinberg, I., & Floyd, T. C. (1979). Systematic trends across the night in human sleep cycles. *Psychophysiology, 16*(3), 283-291.

Feinberg, I., Higgins, L. M., Khaw, W. Y., & Campbell, I. G. (2006). The adolescent decline of NREM delta, an indicator of brain maturation, is linked to age and sex but not to pubertal stage. *Am J Physiol Regul Integr Comp Physiol, 291*(6), R1724-1729.

Finelli, L. A., Borbely, A. A., & Achermann, P. (2001). Functional topography of the human nonREM sleep electroencephalogram. *Eur J Neurosci, 13*(12), 2282-2290.

Frank, M. G., Issa, N. P., & Stryker, M. P. (2001). Sleep enhances plasticity in the developing visual cortex. *Neuron, 30*(1), 275-287.

Franken, P., & Dijk, D. J. (2009). Circadian clock genes and sleep homeostasis. *European Journal of Neuroscience, 29*(9), 1820-1829.

Freund, P. A., & Holling, H. (2011). How to get really smart: Modeling retest and training effects in ability testing using computer-generated figural matrix items. *Intelligence, 39*(4), 233-243.

Fry, A. F., & Hale, S. (1996). Processing Speed, Working Memory, and Fluid Intelligence: Evidence for a Developmental Cascade. *Psychological Science, 7*(4), 237-241.

Fuentealba, P., & Steriade, M. (2005). The reticular nucleus revisited: intrinsic and network properties of a thalamic pacemaker. *Prog Neurobiol, 75*(2), 125-141.

Ghilardi, M., Ghez, C., Dhawan, V., Moeller, J., Mentis, M., Nakamura, T. (2000). Patterns of regional brain activation associated with different forms of motor learning. *Brain Res, 871*(1), 127-145.

Giedd, J. N., Blumenthal, J., Jeffries, N. O., Castellanos, F. X., Liu, H., Zijdenbos, A. (1999). Brain development during childhood and adolescence: a longitudinal MRI study. *Nat Neurosci, 2*(10), 861-863.

Gogtay, N., Giedd, J. N., Lusk, L., Hayashi, K. M., Greenstein, D., Vaituzis, A. C. (2004). Dynamic mapping of human cortical development during childhood through early adulthood. *Proc Natl Acad Sci U S A, 101*(21), 8174-8179.

Gronfier, C., Wright, K. P., Jr., Kronauer, R. E., & Czeisler, C. A. (2007). Entrainment of the human circadian pacemaker to longer-than-24-h days. *Proc Natl Acad Sci U S A, 104*(21), 9081-9086.

Grubb, R. L., Jr., Raichle, M. E., Eichling, J. O., & Ter-Pogossian, M. M. (1974). The effects of changes in PaCO2 on cerebral blood volume, blood flow, and vascular mean transit time. *Stroke, 5*(5), 630-639.

Hausknecht, J. P., Halpert, J. A., Di Paolo, N. T., & Moriarty Gerrard, M. O. (2007). Retesting in selection: a meta-analysis of coaching and practice effects for tests of cognitive ability. *J Appl Psychol, 92*(2), 373-385.

Hebb, D. O. (1949). *The organization of behaviour.* New York: Wiley.

Hering, H., & Sheng, M. (2001). Dendritic spines: structure, dynamics and regulation. *Nat Rev Neurosci, 2*(12), 880-888.

Hernandez, P. J., & Abel, T. (2008). The role of protein synthesis in memory consolidation: progress amid decades of debate. *Neurobiol Learn Mem, 89*(3), 293-311.

Hirshkowitz, M. (2004). Normal human sleep: an overview. *Med Clin North Am, 88*(3), 551-565, vii.

Holmes, J., Gathercole, S. E., & Dunning, D. L. (2009). Adaptive training leads to sustained enhancement of poor working memory in children. *Dev Sci, 12*(4), F9-15.

Holtmaat, A., Wilbrecht, L., Knott, G. W., Welker, E., & Svoboda, K. (2006). Experience-dependent and cell-type-specific spine growth in the neocortex. *Nature, 441*(7096), 979-983.

Horne, J. A. (1993). Human sleep, sleep loss and behaviour. Implications for the prefrontal cortex and psychiatric disorder. *Br J Psychiatry, 162*, 413-419.

Hoshi, Y., Mizukami, S., & Tamura, M. (1994). Dynamic features of hemodynamic and metabolic changes in the human brain during all-night sleep as revealed by near-Infrared spectroscopy. *Brain Research, 652*(2), 257-262.

Huber, R., Ghilardi, M. F., Massimini, M., Ferrarelli, F., Riedner, B. A., Peterson, M. J. (2006). Arm immobilization causes cortical plastic changes and locally decreases sleep slow wave activity. *Nat Neurosci, 9*(9), 1169-1176.

Huber, R., Ghilardi, M. F., Massimini, M., & Tononi, G. (2004). Local sleep and learning. *Nature, 430*(6995), 78-81.

Huber, R., Maatta, S., Esser, S. K., Sarasso, S., Ferrarelli, F., Watson, A. (2008). Measures of cortical plasticity after transcranial paired associative stimulation predict changes in electroencephalogram slow-wave activity during subsequent sleep. *J Neurosci, 28*(31), 7911-7918.

Huber, R., Tononi, G., & Cirelli, C. (2007). Exploratory behavior, cortical BDNF expression, and sleep homeostasis. *Sleep, 30*(2), 129-139.

Hueber, D. M., Fantini, S., Cerussi, A. E., & Barbieri, B. (1999). New optical probe designs for absolute (self-calibrating) NIR tissue hemoglobin measurements. *Optical Tomography and Spectroscopy of Tissue III, Proc. SPIE 3597*, 618-631.

Huttenlocher, P. R. (1979). Synaptic density in human frontal cortex - developmental changes and effects of aging. *Brain Res, 163*(2), 195-205.

Huttenlocher, P. R., & Dabholkar, A. S. (1997). Regional differences in synaptogenesis in human cerebral cortex. *J Comp Neurol, 387*(2), 167-178.

Iadecola, C. (2004). Neurovascular regulation in the normal brain and in Alzheimer's disease. *Nat Rev Neurosci, 5*(5), 347-360.

Iadecola, C., Yang, G., Ebner, T. J., & Chen, G. (1997). Local and propagated vascular responses evoked by focal synaptic activity in cerebellar cortex. *J Neurophysiol, 78*(2), 651-659.

Iber, C., Sonia Ancoli-Israel, P. D., Chesson, A., Quan, S. F., & eds. (2007). *The AASM manual for the scoring of sleep and associated events: rules, terminology and technical specifications* (1 ed.). Westchester, IL: American Acedemy of Sleep Medicine.

Jaeggi, S. M., Buschkuehl, M., Jonides, J., & Perrig, W. J. (2008). Improving fluid intelligence with training on working memory. *Proc Natl Acad Sci U S A, 105*(19), 6829-6833.

Jaeggi, S. M., Buschkuehl, M., Jonides, J., & Shah, P. (2011). Short- and long-term benefits of cognitive training. *Proc Natl Acad Sci U S A, 108*(25), 10081-10086.

Jaeggi, S. M., Buschkuehl, M., Perrig, W. J., & Meier, B. (2010). The concurrent validity of the N-back task as a working memory measure. *Memory, 18*(4), 394-412.

Jaeggi, S. M., Seewer, R., Nirkko, A. C., Eckstein, D., Schroth, G., Groner, R. (2003). Does excessive memory load attenuate activation in the prefrontal cortex? Load-dependent processing in single and dual tasks: functional magnetic resonance imaging study. *Neuroimage, 19*(2 Pt 1), 210-225.

Jaeggi, S. M., Studer-Luethi, B., Buschkuehl, M., Yi-Fen, S., Jonides, J., & Perrig, W. J. (2011). The relationship between n-back performance and matrix reasoning - implications for training and transfer. *Intelligence, 38*(6), 625-635.

Jasper, H. H. (1958). Formal discussion: dendrites. *Electroencephalography and Clinical Neurophysiology. Supplement, 35*(Supp 10), 42-50.

Jedidi, Z., Rikir, E., Muto, V., Mascetti, L., Kusse, C., Foret, A. (2012). Functional neuroimaging of the reciprocal influences between sleep and wakefulness. *Pflugers Arch, 463*(1), 103-109.

Kane, M. J., Conway, A. R., Miura, T. K., & Colflesh, G. J. (2007). Working memory, attention control, and the N-back task: a question of construct validity. *J Exp Psychol Learn Mem Cogn, 33*(3), 615-622.

Kane, M. J., & Engle, R. W. (2003). Working-memory capacity and the control of attention: The contributions of goal neglect, response competition, and task set to Stroop interference. 1. 132

Kane, M. J., Hambrick, D. Z., & Conway, A. R. (2005). Working memory capacity and fluid intelligence are strongly related constructs: comment on Ackerman, Beier, and Boyle (2005). *Psychol Bull, 131*(1), 66-71; author reply 72-65.

Kattler, H., Dijk, D. J., & Borbely, A. A. (1994). Effect of unilateral somatosensory stimulation prior to sleep on the sleep EEG in humans. *J Sleep Res, 3*(3), 159-164.

Kaufmann, C., Wehrle, R., Wetter, T. C., Holsboer, F., Auer, D. P., Pollmacher, T. (2006). Brain activation and hypothalamic functional connectivity during human non-rapid eye movement sleep: an EEG/fMRI study. *Brain, 129*(Pt 3), 655-667.

Klein, D. C., Moore, R. Y., & Reppert, S. M. (1991). *Suprachiasmatic nucleusThe mind's clock*. New York: Oxford University Press, Inc.

Klingberg, T. (2010). Training and plasticity of working memory. *Trends Cogn Sci, 14*(7), 317-324.

Klingberg, T., Fernell, E., Olesen, P. J., Johnson, M., Gustafsson, P., Dahlstrom, K. (2005). Computerized training of working memory in children with ADHD--a randomized, controlled trial. *J Am Acad Child Adolesc Psychiatry, 44*(2), 177-186.

Knoch, D., Gianotti, L. R., Pascual-Leone, A., Treyer, V., Regard, M., Hohmann, M. (2006). Disruption of right prefrontal cortex by low-frequency repetitive transcranial magnetic stimulation induces risk-taking behavior. *J Neurosci, 26*(24), 6469-6472.

Knott, G. W., Holtmaat, A., Wilbrecht, L., Welker, E., & Svoboda, K. (2006). Spine growth precedes synapse formation in the adult neocortex in vivo. *Nat Neurosci, 9*(9), 1117-1124.

Kramer, A., Yang, F. C., Snodgrass, P., Li, X., Scammell, T. E., Davis, F. C. (2001). Regulation of daily locomotor activity and sleep by hypothalamic EGF receptor signaling. *Science, 294*(5551), 2511-2515.

Krauchi, K., & Wirz-Justice, A. (1994). Circadian rhythm of heat production, heart rate, and skin and core temperature under unmasking conditions in men. *Am J Physiol, 267*(3 Pt 2), R819-829.

Kubota, Y., Takasu, N. N., Horita, S., Kondo, M., Shimizu, M., Okada, T. (2011). Dorsolateral prefrontal cortical oxygenation during REM sleep in humans. *Brain Research, 1389*, 83-92.

Kuboyama, T., Hori, A., Sato, T., Mikami, T., Yamaki, T., & Ueda, S. (1997). Changes in cerebral blood flow velocity in healthy young men during overnight sleep and while awake. *Electroencephalography and Clinical Neurophysiology, 102*(2), 125-131.

Kurth, S., Ringli, M., Geiger, A., LeBourgeois, M., Jenni, O. G., & Huber, R. (2010). Mapping of cortical activity in the first two decades of life: a high-density sleep electroencephalogram study. *J Neurosci, 30*(40), 13211-13219.

Kurth, S., Ringli, M., Lebourgeois, M. K., Geiger, A., Buchmann, A., Jenni, O. G. (2012). Mapping the electrophysiological marker of sleep depth reveals skill maturation in children and adolescents. *Neuroimage, 63*(2), 959-965.

Landsness, E. C., Crupi, D., Hulse, B. K., Peterson, M. J., Huber, R., Ansari, H. (2009). Sleep-dependent improvement in visuomotor learning: a causal role for slow waves. *Sleep, 32*(10), 1273-1284.

Lavie, P. (2001). Sleep-wake as a biological rhythm. *Annu Rev Psychol, 52*, 277-303.

Lehrl, S., Gallwitz, A., Blaha, L., & Fischer, B. (1991). *Theorie und Messung der geistigen Leistungsfähigkeit mit dem Kurztest KAI*: Vless.

Li, S. C., Schmiedek, F., Huxhold, O., Rocke, C., Smith, J., & Lindenberger, U. (2008). Working memory plasticity in old age: practice gain, transfer, and maintenance. *Psychol Aging, 23*(4), 731-742.

Liu, C., Weaver, D. R., Strogatz, S. H., & Reppert, S. M. (1997). Cellular construction of a circadian clock: period determination in the suprachiasmatic nuclei. *Cell, 91*(6), 855-860.

Liu, S. Q., & Cull-Candy, S. G. (2000). Synaptic activity at calcium-permeable AMPA receptors induces a switch in receptor subtype. *Nature, 405*(6785), 454-458.

Liu, Z. W., Faraguna, U., Cirelli, C., Tononi, G., & Gao, X. B. (2010). Direct evidence for wake-related increases and sleep-related decreases in synaptic strength in rodent cortex. *J Neurosci, 30*(25), 8671-8675.

Lledo, P. M., Hjelmstad, G. O., Mukherji, S., Soderling, T. R., Malenka, R. C., & Nicoll, R. A. (1995). Calcium/calmodulin-dependent kinase II and long-term potentiation enhance synaptic transmission by the same mechanism. *Proc Natl Acad Sci U S A, 92*(24), 11175-11179.

Logothetis, N. K., & Wandell, B. A. (2004). Interpreting the BOLD signal. *Annual Review of Physiology, 66*, 735-769.

Lopez, J. C. (2002). Quantifying synaptic efficacy. *Nat Rev Neurosci, 3*(5), 332-332.

Luna, B., Garver, K. E., Urban, T. A., Lazar, N. A., & Sweeney, J. A. (2004). Maturation of Cognitive Processes From Late Childhood to Adulthood. *Child Development, 75*(5), 1357-1372.

Lustenberger, C., & Huber, R. (2012). High density electroencephalography in sleep research: potential, problems, future perspective. *Frontiers in Neurology, 3*.

Luthi, A. (2013). Sleep Spindles: Where They Come From, What They Do. *Neuroscientist*.

Maatta, S., Landsness, E., Sarasso, S., Ferrarelli, F., Ferreri, F., Ghilardi, M. F. (2010). The effects of morning training on night sleep: a behavioral and EEG study. *Brain Res Bull, 82*(1-2), 118-123.

Maquet, P. (1995). Sleep function(s) and cerebral metabolism. *Behav Brain Res, 69*(1-2), 75-83.

Maquet, P. (2000). Functional neuroimaging of normal human sleep by positron emission tomography. *Journal of Sleep Research, 9*(3), 207-231.

Maquet, P., Ruby, P., Maudoux, A., Albouy, G., Sterpenich, V., Dang-Vu, T. (2005). Human cognition during REM sleep and the activity profile within frontal and parietal cortices: a reappraisal of functional neuroimaging data. *Progress in Brain Research, 150*, 219-227.

Maret, S., Faraguna, U., Nelson, A. B., Cirelli, C., & Tononi, G. (2011). Sleep and waking modulate spine turnover in the adolescent mouse cortex. *Nature Neuroscience, 14*(11), 1418-1420.

Marshall, L., Helgadottir, H., Molle, M., & Born, J. (2006). Boosting slow oscillations during sleep potentiates memory. *Nature, 444*(7119), 610-613.

Massimini, M., & Amzica, F. (2001). Extracellular calcium fluctuations and intracellular potentials in the cortex during the slow sleep oscillation. *J Neurophysiol, 85*(3), 1346-1350.

Massimini, M., Tononi, G., & Huber, R. (2009). Slow waves, synaptic plasticity and information processing: insights from transcranial magnetic stimulation and high-density EEG experiments. *Eur J Neurosci, 29*(9), 1761-1770.

Matcher, S. J., Cope, M., & Delpy, D. T. (1997). In vivo measurements of the wavelength dependence of tissue-scattering coefficients between 760 and 900 nm measured with time-resolved spectroscopy. *Applied Optics, 36*(1), 386-396.

Matsuzaki, M., Honkura, N., Ellis-Davies, G. C., & Kasai, H. (2004). Structural basis of long-term potentiation in single dendritic spines. *Nature, 429*(6993), 761-766.

Melby-Lervåg, M., & Hulme, C. (2013). Is working memory training effective? A meta-analytic review. *Developmental Psychology, 49*(2), 270-291.

Metz, A. J., Biallas, M., Jenny, C., Muehlemann, T., & Wolf, M. (2013). The effect of basic assumptions on the tissue oxygen saturation value of near infrared spectroscopy. *Advances in Experimental Medicine and Biology, 765*, 169-175.

Metz, A. J., Pugin, F., Huber, R., Achermann, P., & Wolf, M. (2013). Brain tissue oxygen saturation increases during the night in adolescents. *Advances in Experimental Medicine and Biology, 789*, 113-119.

Moore, R. Y., & Eichler, V. B. (1972). Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. *Brain Res, 42*(1), 201-206.

Muehlemann, T., Haensse, D., & Wolf, M. (2008). Wireless miniaturized in-vivo near infrared imaging. *Optics Express, 16*(14), 10323-10330.

Munno, D. W., Prince, D. J., & Syed, N. I. (2003). Synapse number and synaptic efficacy are regulated by presynaptic cAMP and protein kinase A. *J Neurosci, 23*(10), 4146-4155.

Näsi, T., Virtanen, J., Noponen, T., Toppila, J., Salmi, T., & Ilmoniemi, R. J. (2011). Spontaneous hemodynamic oscillations during human sleep and sleep stage transitions characterized with near-infrared spectroscopy. *PLoS ONE, 6*(10), e25415.

Nelson, D. L., Lehninger, A. L., & Cox, M. M. (2008). *Lehninger Principles of Biochemistry* (5 Ed., illustrated ed.). New York: W H Freeman Limited.

Nere, A., Hashmi, A., Cirelli, C., & Tononi, G. (2013). Sleep-dependent synaptic down-selection (I): modeling the benefits of sleep on memory consolidation and integration. *Front Neurol, 4*, 143.

Ngo, H. V., Martinetz, T., Born, J., & Molle, M. (2013). Auditory closed-loop stimulation of the sleep slow oscillation enhances memory. *Neuron, 78*(3), 545-553.

Nichols, T. E., & Holmes, A. P. (2002). Nonparametric permutation tests for functional neuroimaging: A primer with examples. *Human Brain Mapping, 15*(1), 1-25.

Nir, Y., Staba, R. J., Andrillon, T., Vyazovskiy, V. V., Cirelli, C., Fried, I. (2011). Regional slow waves and spindles in human sleep. *Neuron, 70*(1), 153-169.

Nofzinger, E. A., Buysse, D. J., Miewald, J. M., Meltzer, C. C., Price, J. C., Sembrat, R. C. (2002). Human regional cerebral glucose metabolism during non-rapid eye movement sleep in relation to waking. *Brain, 125*(Pt 5), 1105-1115.

Ohayon, M. M., Carskadon, M. A., Guilleminault, C., & Vitiello, M. V. (2004). Meta-analysis of quantitative sleep parameters from childhood to old age in healthy individuals: developing normative sleep values across the human lifespan. *Sleep, 27*(7), 1255-1273.

Olcese, U., Esser, S. K., & Tononi, G. (2010). Sleep and synaptic renormalization: a computational study. *J Neurophysiol, 104*(6), 3476-3493.

Olesen, P. J., Westerberg, H., & Klingberg, T. (2004). Increased prefrontal and parietal activity after training of working memory. *Nat Neurosci, 7*(1), 75-79.

Ott, D. V., Ullsperger, M., Jocham, G., Neumann, J., & Klein, T. A. (2011). Continuous theta-burst stimulation (cTBS) over the lateral prefrontal cortex alters reinforcement learning bias. *Neuroimage, 57*(2), 617-623.

Owen, A. M., Hampshire, A., Grahn, J. A., Stenton, R., Dajani, S., Burns, A. S. (2010). Putting brain training to the test. *Nature, 465*(7299), 775-778.

Owen, A. M., McMillan, K. M., Laird, A. R., & Bullmore, E. (2005). N-back working memory paradigm: a meta-analysis of normative functional neuroimaging studies. *Hum Brain Mapp, 25*(1), 46-59.

Pascual-Leone, A., Amedi, A., Fregni, F., & Merabet, L. B. (2005). The plastic human brain cortex. *Annu Rev Neurosci, 28*, 377-401.

Passolunghi, M. C., & Mammarella, I. C. (2012). Selective spatial working memory impairment in a group of children with mathematics learning disabilities and poor problem-solving skills. *J Learn Disabil, 45*(4), 341-350.

Petermann, F., & Petermann, U. (2007). *Hamburg-Wechsler-Intelligenztest für Kinder - IV (HAWIK)* (4th ed.). Bern: Huber.

Pizza, F., Biallas, M., Wolf, M., Werth, E., & Bassetti, C. L. (2010). Nocturnal cerebral hemodynamics in snorers and in patients with obstructive sleep apnea: a near-infrared spectroscopy study. *Sleep, 33*(2), 205-210.

Pugin, F., Metz, A. J., Stauffer, M., Wolf, M., Jenni, O. G., & Huber, R. (under review). Working memory training shows immediate and long-term effects on cognitive performance in children and adolescents.

Raichle, M. E., & Mintun, M. A. (2006). Brain work and brain imaging. *Annu Rev Neurosci, 29*, 449-476.

Rechtschaffen, A., & Kales, A. (1968). *A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects*: U.S Department of Health, Education and Welfare Public Health Service, National Institutes of Health, National Institute of Neurological Diseases and Blindness, Neurological Information Network.

Redick, T. S., Shipstead, Z., Harrison, T. L., Hicks, K. L., Fried, D. E., Hambrick, D. Z. (2013). No evidence of intelligence improvement after working memory training: a randomized, placebo-controlled study. *J Exp Psychol Gen, 142*(2), 359-379.

Riedner, B. A., Vyazovskiy, V. V., Huber, R., Massimini, M., Esser, S., Murphy, M. (2007). Sleep homeostasis and cortical synchronization: III. A high-density EEG study of sleep slow waves in humans. *Sleep, 30*(12), 1643-1657.

Ringli, M., Souissi, S., Kurth, S., Brandeis, D., Jenni, O. G., & Huber, R. (2012). Topography of sleep slow wave activity in children with attention-deficit/hyperactivity disorder. *Cortex*.

Roberts, P. D., & Bell, C. C. (2002). Spike timing dependent synaptic plasticity in biological systems. *Biol Cybern, 87*(5-6), 392-403.

Roy, C. S., & Sherrington, C. S. (1890). On the Regulation of the Blood-supply of the Brain. *The Journal of Physiology, 11*(1-2), 158-157-158-117.

Sanchez-Vives, M. V., & McCormick, D. A. (2000). Cellular and network mechanisms of rhythmic recurrent activity in neocortex. *Nat Neurosci, 3*(10), 1027-1034.

Saper, C. B., Chou, T. C., & Scammell, T. E. (2001). The sleep switch: hypothalamic control of sleep and wakefulness. *Trends Neurosci, 24*(12), 726-731.

Saper, C. B., Scammell, T. E., & Lu, J. (2005). Hypothalamic regulation of sleep and circadian rhythms. *Nature, 437*(7063), 1257-1263.

Sarasso, S., Maatta, S., Ferrarelli, F., Poryazova, R., Tononi, G., & Small, S. L. (2014). Plastic changes following imitation-based speech and language therapy for aphasia: a high-density sleep EEG study. *Neurorehabil Neural Repair, 28*(2), 129-138.

Scholkmann, F., Gerber, U., Wolf, M., & Wolf, U. (2012). End-tidal CO(2): An important parameter for a correct interpretation in functional brain studies using speech tasks. *Neuroimage, 66C*, 71-79.

Scholkmann, F., Spichtig, S., Muehlemann, T., & Wolf, M. (2010). How to detect and reduce movement artifacts in near-infrared imaging using moving standard deviation and spline interpolation. *Physiological Measurement, 31*(5), 649-662.

Seitz, R. J., & Roland, P. E. (1992). Vibratory stimulation increases and decreases the regional cerebral blood flow and oxidative metabolism: a positron emission tomography (PET) study. *Acta Neurol Scand, 86*(1), 60-67.

Shaw, P., Greenstein, D., Lerch, J., Clasen, L., Lenroot, R., Gogtay, N. (2006). Intellectual ability and cortical development in children and adolescents. *Nature, 440*(7084), 676-679.

Shaw, P., Kabani, N. J., Lerch, J. P., Eckstrand, K., Lenroot, R., Gogtay, N. (2008). Neurodevelopmental trajectories of the human cerebral cortex. *J Neurosci, 28*(14), 3586-3594.

Shiotsuka, S., Atsumi, Y., Ogata, S., Yamamoto, R., Igawa, M., Takahashi, K. (1998). Cerebral blood volume in the sleep measured by near-infrared spectroscopy. *Psychiatry and Clinical Neurosciences, 52*(2), 172-173.

Shipstead, Z., Redick, T. S., & Engle, R. W. (2012). Is working memory training effective? *Psychol Bull, 138*(4), 628-654.

Shore, E. T., Millman, R. P., Silage, D. A., Chung, D. C., & Pack, A. I. (1985). Ventilatory and arousal patterns during sleep in normal young and elderly subjects. *Journal of Applied Physiology, 59*(5), 1607-1615.

Smith, E. E., & Jonides, J. (1999). Storage and executive processes in the frontal lobes. *Science, 283*(5408), 1657-1661.

Soderling, T. R., & Derkach, V. A. (2000). Postsynaptic protein phosphorylation and LTP. *Trends Neurosci, 23*(2), 75-80.

Spielman, A. J., Zhang, G., Yang, C. M., D'Ambrosio, P., Serizawa, S., Nagata, M. (2000). Intracerebral hemodynamics probed by near infrared spectroscopy in the transition between wakefulness and sleep. *Brain Research, 866*(1-2), 313-325.

Stadelmann, K., Latshang, T. D., Lo Cascio, C. M., Tesler, N., Stoewhas, A. C., Kohler, M. (2013). Quantitative changes in the sleep EEG at moderate altitude (1630 m and 2590 m). *PLoS One, 8*(10), e76945.

Steriade, M. (2006). Grouping of brain rhythms in corticothalamic systems. *Neuroscience, 137*(4), 1087-1106.

Steriade, M., McCormick, D. A., & Sejnowski, T. J. (1993). Thalamocortical oscillations in the sleeping and aroused brain. *Science, 262*(5134), 679-685.

Steriade, M., Nunez, A., & Amzica, F. (1993a). Intracellular analysis of relations between the slow (< 1 Hz) neocortical oscillation and other sleep rhythms of the electroencephalogram. *J Neurosci, 13*(8), 3266-3283.

Steriade, M., Nunez, A., & Amzica, F. (1993b). A novel slow (< 1 Hz) oscillation of neocortical neurons in vivo: depolarizing and hyperpolarizing components. *J Neurosci, 13*(8), 3252-3265.

Steriade, M., & Timofeev, I. (2003). Neuronal plasticity in thalamocortical networks during sleep and waking oscillations. *Neuron, 37*(4), 563-576.

Steriade, M., Timofeev, I., & Grenier, F. (2001). Natural waking and sleep states: a view from inside neocortical neurons. *J Neurophysiol, 85*(5), 1969-1985.

Stins, J. F., Polderman, J. C., Boomsma, D. I., & de Geus, E. J. (2007). Conditional accuracy in response interference tasks: Evidence from the Eriksen flanker task and the spatial conflict task. *Adv Cogn Psychol, 3*(3), 409-417.

Strangman, G., Boas, D. A., & Sutton, J. P. (2002). Non-invasive neuroimaging using near-infrared light. *Biol Psychiatry, 52*(7), 679-693.

Tachtsidis, I., Tisdall, M. M., Leung, T. S., Pritchard, C., Cooper, C. E., Smith, M. (2009). Relationship between brain tissue haemodynamics, oxygenation and metabolism in the healthy human adult brain during hyperoxia and hypercapnea. *Advances in Experimental Medicine and Biology, 645*, 315-320.

Thorell, L. B., Lindqvist, S., Bergman Nutley, S., Bohlin, G., & Klingberg, T. (2009). Training and transfer effects of executive functions in preschool children. *Dev Sci, 12*(1), 106-113.

Timofeev, I., & Chauvette, S. (2011). Thalamocortical Oscillations: Local Control of EEG Slow Waves. *Current Topics in Medicinal Chemistry, 11*(19), 2457.

Timofeev, I., & Steriade, M. (1996). Low-frequency rhythms in the thalamus of intact-cortex and decorticated cats. *J Neurophysiol, 76*(6), 4152-4168.

Tobler, I. (2000). Phylogeny of sleep regulation. In M. H. Kryger, T. Roth & W. C. Dement (Eds.), *Principles and practice of sleep medicine*. Philadelphia: W.B. Saunders Co.

Tononi, G., & Cirelli, C. (2006). Sleep function and synaptic homeostasis. *Sleep Medicine Reviews, 10*(1), 49-62.

Trachtenberg, J. T., Chen, B. E., Knott, G. W., Feng, G., Sanes, J. R., Welker, E. (2002). Long-term in vivo imaging of experience-dependent synaptic plasticity in adult cortex. *Nature, 420*(6917), 788-794.

Turrigiano, G. G. (1999). Homeostatic plasticity in neuronal networks: the more things change, the more they stay the same. *Trends Neurosci, 22*(5), 221-227.

Turrigiano, G. G., Leslie, K. R., Desai, N. S., Rutherford, L. C., & Nelson, S. B. (1998). Activity-dependent scaling of quantal amplitude in neocortical neurons. *Nature, 391*(6670), 892-896.

Uchida-Ota, M., Tanaka, N., Sato, H., & Maki, A. (2008). Intrinsic correlations of electroencephalography rhythms with cerebral hemodynamics during sleep transitions. *Neuroimage, 42*(1), 357-368.

Unsworth, N., Redick, T. S., Heitz, R. P., Broadway, J. M., & Engle, R. W. (2009). Complex working memory span tasks and higher-order cognition: a latent-variable analysis of the relationship between processing and storage. *Memory, 17*(6), 635-654.

Virtanen, J., Noponen, T., Kotilahti, K., & Ilmoniemi, R. J. (2011). Accelerometer-based method for correcting signal baseline changes caused by motion artifacts in medical near-infrared spectroscopy. *Journal of Biomedical Optics, 16*(8), 087005.

Vyazovskiy, V. V., Cirelli, C., Pfister-Genskow, M., Faraguna, U., & Tononi, G. (2008). Molecular and electrophysiological evidence for net synaptic potentiation in wake and depression in sleep. *Nat Neurosci, 11*(2), 200-208.

Vyazovskiy, V. V., & Harris, K. D. (2013). Sleep and the single neuron: the role of global slow oscillations in individual cell rest. *Nat Rev Neurosci, 14*(6), 443-451.

Vyazovskiy, V. V., Olcese, U., Hanlon, E. C., Nir, Y., Cirelli, C., & Tononi, G. (2011). Local sleep in awake rats. *Nature, 472*(7344), 443-447.

Vyazovskiy, V. V., Olcese, U., Lazimy, Y. M., Faraguna, U., Esser, S. K., Williams, J. C. (2009). Cortical firing and sleep homeostasis. *Neuron, 63*(6), 865-878.

Vyazovskiy, V. V., Riedner, B. A., Cirelli, C., & Tononi, G. (2007). Sleep homeostasis and cortical synchronization: II. A local field potential study of sleep slow waves in the rat. *Sleep, 30*(12), 1631-1642.

Wang, S., & Gathercole, S. E. (2013). Working memory deficits in children with reading difficulties: memory span and dual task coordination. *J Exp Child Psychol, 115*(1), 188-197.

Werth, E., Dijk, D. J., Achermann, P., & Borbely, A. A. (1996). Dynamics of the sleep EEG after an early evening nap: experimental data and simulations. *Am J Physiol, 271*(3 Pt 2), R501-510.

Westerberg, H., & Klingberg, T. (2007). Changes in cortical activity after training of working memory--a single-subject analysis. *Physiol Behav, 92*(1-2), 186-192.

Wilhelm, I., Ringli, M., Kurth, S., Geiger, A., Jenni, O. G., & Huber, R. (2013). Use-dependent changes in local slow wave activity in three different age-groups. *Abstract, 21. Tagung Fachgruppe Entwicklungspsychologie* (http://www.epsy2013.de/docs/Abstractband_EPSY2013.pdf).

Wolf, M., Morren, G., Haensse, D., Karen, T., Wolf, U., Fauchere, J. C. (2008). Near infrared spectroscopy to study the brain: an overview. *Opto-Electronics Review, 16*(4), 413-419.

Wyatt, J. K., Ritz-De Cecco, A., Czeisler, C. A., & Dijk, D. J. (1999). Circadian temperature and melatonin rhythms, sleep, and neurobehavioral function in humans living on a 20-h day. *Am J Physiol, 277*(4 Pt 2), R1152-1163.

Xu, T., Yu, X., Perlik, A. J., Tobin, W. F., Zweig, J. A., Tennant, K. (2009). Rapid formation and selective stabilization of synapses for enduring motor memories. *Nature, 462*(7275), 915-919.

Yang, G., Pan, F., & Gan, W.-B. (2009). Stably maintained dendritic spines are associated with lifelong memories. *Nature, 462*(7275), 920-924.

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9. Publication list

Articles

Pugin, F., Metz, A.J., Wolf, M., Achermann, P, Jenni, O.G., Huber, R.
(under review)
Local increase of slow wave activity after three weeks of working memory training in children and adolescents

Pugin, F., Metz, A.J., Stauffer, M., Wolf, M., Jenni, O.G., Huber, R. (under review)
Working memory training shows immediate and long-term effects on cognitive performance in children and adolescents

Metz, A.J., Pugin, F., Huber, R., Achermann, P., Wolf, M. (2014)
Changes of cerebral tissue oxygen saturation at sleep transitions in adolescents.
Advances in Experimental Medicine and Biology

Lustenberger, C., O'Gorman, R.L., Pugin, F., Tüshaus, L., Wehrle, F., Achermann, P., Huber, R. (2014)
Sleep spindles are related to schizotypal personality traits and thalamic glutamine/glutamate in healthy subjects
Schizophrenia Bulletin

Metz, A.J., Pugin, F., Huber, R., Achermann, P., Wolf, M. (2013)
Brain tissue oxygen saturation increases during the night in adolescents
Advances in Experimental Medicine and Biology

Viola, A.U., Chellappa, S.L., Archer, S.N., Pugin, F., Götz, T., Dijk, D.-J., Cajochen, Ch. (2011)
Interindividual differences in circadian rhythmicity and sleep homeostasis in older people: effect of a PER3 polymorphism
Neurobiology of Aging

Oral presentations

Hirntraining beeinflusst Tiefschlaf und begünstigt Intelligenz

Symposium Forschungszentrum für das Kind (FZK) / Children's Research Center (CRC) Retreat

University Children's Hospital Zurich, Switzerland (2013)

The effects of an intensive cognitive training on cognition and sleep EEG topography

Colloquium Current Topics in Sleep and Chronobiology Research (2013)

University of Zurich, Switzerland

The effect of an intensive cognitive training on sleep EEG topography and cognition

4th Retreat of the Child Development Center

Au (Zurich), Switzerland (2013)

The effect of an intensive cognitive training on sleep EEG topography and cognition

2nd Children's Research Center (CRC) Retreat / Au (Zurich), Switzerland Forschungszentrum für das Kind (FZK) (2012)

Awarded for outstanding presentation

Poster presentations

Pugin, F., Metz, A.J., Stauffer, M., Rauch, A., Jäncke, L., Achermann, P., Wolf, M., Jenni, O., Huber, R. (2013)

Local sleep EEG topography is associated with training induced working memory performance increase

3rd Children's Research Center (CRC) Retreat / Forschungszentrum für das Kind (FZK)

Au (Zurich), Switzerland

Pugin, F., Metz, A.J., Stauffer, M., Rauch, A., Jäncke, L., Achermann, P., Wolf, M., Jenni O., Huber, R. (2013)

An intensive cognitive training in children: Effects on cognitive performance and sleep spindle EEG topography in children

9th Symposium of the Zurich Center for Integrative Human Physiology (ZIHP)

Zurich, Switzerland

Pugin, F., Metz, A.J., Stauffer, M., Rauch, A., Jäncke, L., Achermann, P., Wolf, M., Jenni O., Huber, R. (2013)

The effects of intensive cognitive training on cognitive performance and sleep EEG topography in children

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Pugin, F., Metz, A.J., Stauffer, M., Rauch, A., Jäncke, L., Achermann, P., Wolf, M., Jenni O., Huber, R. (2013)

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Swiss Society for Neuroscience (SSN)

Geneva, Switzerland

Pugin, F., Metz, A.J., Stauffer, M., Rauch, A., Jäncke, L., Achermann, P., Wolf, M., Jenni O., Huber, R. (2012)

The effects of intensive cognitive training on cognitive performance and sleep EEG topography in children

21st Congress of the European Sleep Research Society (ESRS)

Paris, France

Pugin, F., Metz, A.J., Stauffer, M., Rauch, A., Jäncke, L., Achermann, P., Wolf, M., Jenni O., Huber, R. (2012)

The effects of intensive cognitive training on cognitive performance and sleep EEG topography in young male

8th Symposium of the Zurich Center for Integrative Human Physiology (ZIHP)

Zurich, Switzerland

Pugin, F., Metz, A.J., Stauffer, M., Rauch, A., Jäncke, L., Achermann, P., Wolf, M., Jenni O., Huber, R. (2012)

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Pugin, F., Metz, A.J., Stauffer, M., Rauch, A., Jäncke, L., Achermann, P., Wolf, M., Jenni O., Huber, R. (2012)

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